THESIS

INTERACTIVE EFFECTS OF *MELOIDOGYNE INCOGNITA* AND *SCLEROTINIA SCLEROTIORUM* ON *MENTHA ARVENSIS* AND THEIR MANAGEMENT

SUMMARY

THESIS

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BY

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THESIS

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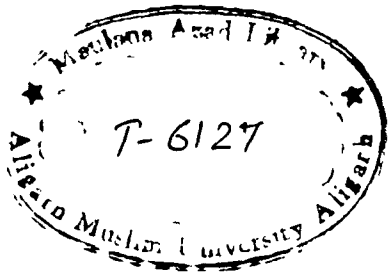


SUMMARY

Japanese mint, *Mentha arvensis* L. is a rich source of menthol, a chemical, which is widely used in pharmaceutical, flavouring and cosmetic industries. It is cultivated on a large scale in tropical and sub-tropical countries of the world. Besides other pathogens, fungi and plant-parasitic nematodes causes considerable damage to it. Keeping in view the importance of *M. arvensis*, an ever-increasing demand for menthol, and the damaging potential of root-knot nematodes alone and in combination with several soil borne fungi, attempts were made to generate information pertaining to survey of mint growing areas in Uttar Pradesh for the association of plant-parasitic nematodes and soil borne fungi infecting Japanese mint, singular and combined effect of *Meloidogyne incognita* and *Sclerotinia sclerotiorum* on growth and oil yield of *M. arvensis* cv. Gomti, factors effecting (soil type and pH) the severity of disease and measures to manage the losses due to these pathogens.

Survey carried out in districts of Aligarh, Badaun, Bareilly, Bulandshahar, Etah, Moradabad and Rampur indicated that during initial stages of plant growth in March, visually there were no specific symptoms of nematode attack. The water soaked areas on suckers due to *S. sclerotiorum* were frequently noticed. At crop maturity in May, patches of diseased plants showed symptoms of stunting with chlorotic and smaller leaves. Roots/suckers of such plants had severe galling with shiny egg masses. At several places, the roots/suckers were dark brown to black in colour and many were rotting. At various locations black coloured sclerotia of *S. sclerotiorum* were also found attached to the infected suckers.

In general, *Meloidogyne* spp. J2 was the dominant population in the rhizosphere of *M. arvensis*. However, in many samples either

Tylenchorhynchus spp., *R. reniformis* or *P. thornei* dominated the population. Among other plant-parasitic nematodes, *Hel. indicus* and *Hop. indicus* were found consistently in higher numbers, while *Tylenchus* sp., *Xiphinema* sp., *Longidorous* sp. and *Criconimoides* sp. were found occasionally. On the examination of perineal patterns of mature females excised from the roots/suckers of *M. arvensis,* collected from each and every locality during survey revealed that *M. incognita* (70%) was more prevalent than *M. javanica* (30%).

At all localities roots/suckers of *M. arvensis* were mainly infected with *S. sclerotiorum*, though in some cases other soil borne fungi were also found infecting the roots/suckers of *M. arvensis.* Fungi isolated from infected roots and suckers were *Fusarium pallidoroseum, F. solani* and *Rhizoctonia solani, S. sclerotiorum.* The highest population of *Meloidogyne* spp., root-knot index and percent root/suckers infection by fungi was found in Moradabad followed by Bulanshahar, Aligarh, Badaun, Bareilly, Rampur and Etah districts, respectively.

The pathogenicity tests of *M. incognita* on *M. arvensis* cv. Gomti evidenced its potentiality in reducing the shoot height, shoot-root/sucker fresh and dry weights, oil yield, total chlorophyll, total phenol and total sugar content of fresh leaves. In general there was a positive relationship between the initial inoculum levels (Pi) of *M. incognita* and reduction in all the test parameters. The maximum reduction in corresponding parameters was 43.4, 45.0, 48.9, 45.7, 49.6, 42.5, 45.5, 47.0 and 45.9%, respectively, at the highest initial inoculum level (25,000 J2/5 kg soil) as compared to uninoculated control. There was a negative relationship between initial inoculum densities and rate of nematode multiplication (Rf). Maximum nematode final population (Pf) (1,33,430) and root-knot index (3.00) were

observed at the highest Pi (25,000 J2/5 kg soil), whereas, maximum Rf (81.18) was observed at minimum Pi (500 J2/5 kg soil).

Increasing inoculum levels of *S. sclerotiorum* also exhibited a gradual increase in extent of reduction in, shoot height, shoot, roots/suckers, fresh and dry weight, oil yield, chlorophyll, total phenol and total sugar content of *M. arvensis* cv. Gomti, and increase in the percent roots/suckers infection by the fungus. The maximum reduction in the corresponding test parameters was 30.4, 39.8, 43.6, 40.3, 42.9, 28.9, 31.4, 34.8 and 31.6%, respectively at the highest initial inoculum level of 12 g fungal mycelium/5 kg soil as compared to uninoculated control. At the lowest Pi (1 g mycelium/5 kg soil), infection was observed 18.0% and at the highest Pi (12 g mycelium/5 kg soil), it was 80.2%.

The sequential, simultaneous and single inoculation of *M. incognita* (5000 J2/5 kg soil) and *S. sclerotiorum* (3 g mycelium/5 kg soil) on *M. arvensis* cv. Gomti indicates that the highest reduction in plant growth, and plant chemicals measured were found in plants inoculated with the nematode and fungus simultaneously followed by nematode inoculated seven days prior to fungus and fungus inoculated seven days prior to nematode, respectively. The maximum reduction in shoot height (32.6%), shoot dry weight (48.2%), root/sucker dry weight (51.5%), oil yield (33.3%), total chlorophyll (36.3%), total phenol (37.2%) and total sugar (32.3%) was observed in simultaneous nematode and fungus inoculation. Highest reproduction rate (19.71) of *M. incognita* and root-knot index (1.90) were observed in plants inoculated with the nematode alone, whereas, highest roots/suckers infection by the fungus (62.0%) was observed in plants inoculated simultaneously with the nematode and the fungus.

The effect of different pH levels (4.5, 6.0, 7.5, 9.5) on plants inoculated with nematode alone and nematode and fungus simultaneously showed maximum reduction in shoot height, shoot, root/sucker, fresh and dry weight, oil yield, chlorophyll, total sugar and total phenol content at pH 9.0 followed by 7.5, 6.0 and 4.5, respectively, whereas, the plants inoculated with fungus alone showed maximum reduction in corresponding parameters at pH 4.5 followed by 6.0, 7.5 and 9.0 respectively. The highest reproduction rate and root-knot index were observed at pH 9.0 followed by 7.5, 6.0 and 4.5, respectively. The roots/suckers infection due to *S. sclerotiorum* was found to be highest at pH 4.5 followed by pH 6.0, 7.5 and 9.0, respectively.

The effect of different soil types on various plant growth parameters of *M. arvensis* cv. Gomti was significant, both in the absence and presence of the nematode and the fungus. The highest reduction in plant growth and oil yield was observed in loamy sand soil followed by sandy loam, sandy clay loam and sandy clay, respectively as compared to uninoculated plants. The highest nematode population, reproduction rate, root-knot index was observed in loamy sand followed by sandy loam, sandy clay loam and sandy clay respectively.

The various treatments, viz. carbofuran (1.5 mg a.i./kg soil), carbendazim (1.0 mg a.i./kg soil), neem seed powder (50 mg/kg soil), *P. lilacinus/T. harzianum* (50 mg culture/kg soil) and *P. fluorescens* (50 mg/kg soil) used alone and in different combinations (half of the standard dose) resulted an increase in the growth of *M. arvensis* cv. Gomti in comparison to untreated inoculated plants. Greatest improvement in plant growth and reduction in reproduction of *M. incognita* was achieved in plants treated with neem seed powder + carbofuran and in descending order carbofuran alone, neem seed powder alone, carbofuran +

carbendazim, *P. lilacinus* + neem seed powder, *T. harzianum* + neem seed powder, *P. fluorescencs* + neem seed powder, neem seed powder + carbendazim, *P. lilacinus* + *T. harzianum*, *P. fluorescencs* + *P. lilacinus*, *P. fluorescencs* + *T. harzianum*, *P. lilacinus* alone, *T. harzianum* alone, *P. fluorescencs* alone and carbendazim alone, respectively

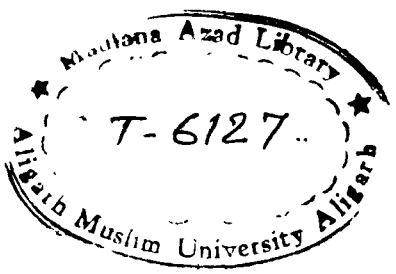
All the treatments, viz. carbofuran (1.5 mg a.i./kg soil), carbendazim (1.0 mg a.i./kg soil), neem seed powder (50 mg/kg soil), *P. lilacinus/T. harzianum* (50 mg culture/kg soil) and *P. fluorescens* (50 mg/kg soil) used alone and in different combinations (half of the standard dose) against *S. sclerotiorum* revealed that all the treatments were able to boost the plant growth and provided the satisfactory reduction in disease incidence. The greatest plant growth and lowest root/suckers infection was observed in the plants treated with neem seed powder + carbendazim, followed by carbendazim alone, neem seed powder alone, carbofuran + carbendazim, neem seed powder + *T. harzianum*, neem seed powder + *P. fluorescens*, neem seed powder + *P. lilacinus*, neem seed powder + carbofuran, *P. fluoresens* + *T. harzianum*, *P. lilacinus* + *T. harzianum*, *P. fluorescens* + *P. lilacinus*, *T. harzianum* alone, *P. fluorescens* alone, *P. lilacinus* alone and carbofuran alone, respectively.

The management of *M. arvensis* cv. Gomti inoculated with *M. incognita* and *S. sclerotiorum* simultaneously showed that all treatments were able to improve the plant growth and oil yield as compared to untreated inoculated plants. These treatments were also able to reduce the root-knot disease development, nematode reproduction rate and roots and suckers infection due to the fungus. The most satisfactory result were achieved by the neem seed powder (50 kg/ha) + carbofuran (1.5 kg a.i./ha), carbofuran (1.5 kg a.i./ha) + carbendazim (1.0 kg a.i./ha), neem seed powder (50 kg/ha) + carbendazim (1.0 kg a.i./ha).

The investigation conducted for the management of *M. arvensis* cv. Gomti in the field infested with *M. incognita* and *S. sclerotiorum* showed the positive efficacy of all the treatments against the disease complex. The greatest increase in shoot weight, oil yield and highest reduction in root-knot disease development, roots and suckers infection due to the fungus were provided by neem seed powder (50 kg/ha) + carbofuran (1.5 kg a.i./ha), carbofuran (1.5 kg a.i./ha) + carbendazim (1.0 kg a.i./ha), neem seed powder (50 kg/ha) + carbendazim (1.0 kg a.i./ha), carbofuran alone (3.0 kg a.i./ha), and neem seed powder alone (100 kg/ha).

The studies carriedout under the Ph.D. programme have generated knowledge on the occurrence of *M. incognita-S. sclerotiorum* disease complex of *M. arvensis*, effect of soil factors on disease development and effective management options. This information is not only of academic importance but also highly useful for mint farmers. On the basis studies, it is concluded that farmers should have information of their field soil infestation with pathogens, transplant disease-free suckers in main-field and if needed may adopt proper management options.

On the basis of the studies, application of neem seed powder (50 kg/ha) + carbofuran (1.5 kg a.i./ha) or carbendazim (1.0 kg a.i./ha) may be recommended for getting best results towards the management of *M. incognita-S. sclerotiorum* disease complex. However, to avoid the use of pesticides, application of neem seed powder (100 kg/ha) or neem seed powder (50 kg/ha) + *T. harzianum/P. lilacinus/P. fluorescens* (50 kg /ha) may be recommended.



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Certificate

This is to certify that the work embodied in the thesis entitled **'Interactive effects of *Meloidogyne incognita* and *Sclerotinia sclerotiorum* on *Mentha arvensis* and their management'** is the original research work carried out by Ms. Kahkashan Perveen under my supervision. She has fulfilled the prescribed conditions given in the ordinance and regulations of Aligarh Muslim University, Aligarh, India.

I further certify that the work of thesis, either partially or fully, has not been submitted to any other University or Institution for the award of any other degree or diploma.

16-2-2004

(Prof. Akhtar Haseeb)

Dedicated

to my

ever loving

Mummy-Papa

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Kahkashan Perveen.

(Kahkashan Perveen)

LIST OF ABBREVIATIONS

µ	Micro
Abstr.	Abstract
A.D.	*anno Domini*
a.i	Active ingredient
A.M.U.	Aligarh Muslim University
ap	Apothecia
B	Boron
BOD	Biological Oxygen Demand
Ca	Calcium
cfu	Colony forming units
chl	Chlorophyll
CIMAP	Central Institute of Medicinal and Aromatic Plants
Cl	Chlorine
cm	Centimeter
Cu	Copper
DD	Dichloropropene and dichloropropane
EC	Emulsifiable concentration
EDB	Ethylene dibromide
Fe	Iron
Fe-EDTA	Ferric ethylene diamine tetracetate
Fig.	Figure
g	Gram
h	Hour
ha	Hectare
HCl	Hydro chloride
IAA	Indole acetic Acid
IARI	Indian Agricultural Research Institute
ITCC	Indian Type Culture Collection
J2	Second stage juvenile
K	Potassium
kg	Kilogram
l	Liter
lb	Pound
LSD	Least Standard Deviation
m	Meter
Mg	Magnesium
ml	Milliliter
Mn	Manganese
Mo	Molebedate
my	Mycelium
N	Nitrogen

Na_2CO_3	Sodium carbonate
NaOH	Sodium hydroxide
nm	Nano meter
NO_3	Nitrate
O.D.	Optical Density
°C	Degree Celsius
P	Phosphorus
PDA	Potato Dextrose Agar
PDB	Potato Dextrose Broth
Pf	Final population
PGPR	Plant growth promoting rhizo-bacteria
pH	The logarithm to base 10 of the reciprocal of the concentration of hydrogen ion
Pi	Initial population
ppm	Parts per million
q	Quintal
RCI	Root colonization index
Rf	Reproduction factor
RKI	Root-knot index
rpm	Revolution per minute
S	Sulphar
Sc	Sclerotia
t	Ton
U.P.	Uttar Pradesh
WP	Wettable powder
Zn	Zinc

Chapter 1

Introduction

INTRODUCTION

In recent years, there has been an increased interest in the cultivation of medicinal and aromatic plants to meet the requirements of pharmaceuticals, flavouring and perfumery industries. Mints (*Mentha* spp., family Lamiaceae) have been grown and utilized since ancient times and are believed to have originated in the Mediterranean basin and spread to rest of the world. Distillation of mint oil was mentioned even in 410 A.D. in an account written by Synesius of Alexandria, the Bishop of Ptolemais. Spearmint (*Mentha spicata* L.) was first to be cultivated in convent gardens about the 9th century. John Ray first described peppermint (*Mentha piperita* L.) in 1696 in England. The commercial cultivation of mint moved from England to several European countries and the U.S.A. by 1800 A.D. Japanese mint (*Mentha arvensis* L.) was introduced in Japan from China in 1700 A.D. By 1892, mint oil was considered as an outstanding stomachie, stimulant, antispasmodic and carminative.

Mints have been cultivated on a large scale in many tropical countries of the world including Brazil, China, India, Japan and U.S.A. These mints were introduced in India during 1954 at the Regional Research Laboratory, Jammu (Husain *et al.*, 1978). Since then, as a result of the efforts made by the Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow and Essential Oil Association of India, India is now among the leading mint oil exporting countries (George, 1994; Bhattacharya, 1998).

The area with most commercial cultivation of mint has been in Uttar Pradesh and Uttaranchal but during the past few years, it has spread throughout the country especially in north and northwestern states including Madhya Pradesh. Among different species of mint, *M. arvensis* is under large-scale cultivation followed by *M. piperita, M. spicata* and *M. citrata,* respectively, whereas *M. viridis* (Podina) is grown in kitchen gardens.

Japanese mint yielding essential oil on hydro-distillation from fresh herb is a rich source of menthol, a chemical, which is widely used in pharmaceutical, flavoring and cosmetic industries. The oil of peppermint, which contains less

menthol but has a sweeter aroma and taste, is used in toothpastes, chewing-gums, candies, high-grade liquors, medicines and other pharmaceutical preparations. Spearmint oil is mainly used for flavoring. Whereas, Bergamot mints oil is mainly used in perfumery and flavoring due to high content of linalyl acetate and linalool. Average oil content and composition in fresh herb of different species of *Mentha* is listed below:

Mentha species	Oil content of fresh herb (%)	Major components in oil (%)
M. arvensis	0.75 – 0.90	Menthol (80-90)
M. cardiaca	0.40 – 0.55	Carvone (65-80)
M. citrata	0.45 – 0.65	Linalool (35-53), Linalyl acetate (45-60)
M. piperita	0.40 – 0.55	Menthol (40-50), Menthone (20-30), Iso-Menthone (25-30)
M. spicata	0.50 – 0.60	Limonene (25-35), Carvone (60-75)

Source: Haseeb and Shukla, 2001a

Cultivation of mint crops has increased tremendously due to economic returns of the oil and its components. The cultivation of different mints, however, is affected by a number of diseases caused by insects, fungi, viruses and plant-parasitic nematodes (Ikata, 1930; Buhrer, 1938; Horner and Jensen, 1954; Bergeson and Green, 1979; Sattar *et al.*, 1979; Haseeb and Pandey, 1989; Haseeb, 1992, 1994; Singh and Haseeb, 1998; Haseeb and Shukla, 2000a, 2001a).

Plant-Parasitic Nematodes

Nematodes are not only a diverse group of animals, but like bacteria, viruses and insects also occupy all biotopes. Despite their low evolutionary status, nematodes live in habitats, ranging from soil, fresh water and oceans to tall mountains and to the floor of the Arctic and Antarctic. They are present in unimaginable numbers (one million/m^2 soil). A single acre of soil from arable land may contain as many as $3x10^9$ nematodes, while marine beach sand may contain $15x10^8$ per acre. The estimate of existing species of nematodes is around $5x10^5$ or more, mostly parasitizing vertebrates, invertebrates, like molluscs,

crustaceans, insects, centipedes, millipedes, annelids, free living aquatic/ marine/fresh water, soil inhabiting and plant-parasitic species (Jairajpuri, 1988).

Plant-parasitic nematodes constitute only 10 per cent of the total species. A plant may be attacked by one or more species at a time during the period of growth. Nematode may be found attacking roots, stems, leaves and seeds of host plants (Goodey *et al.*, 1965; Webster, 1972; Lamberti and Taylor, 1979; Sitaramaiah, 1984). They produce disease symptoms on susceptible hosts and reduce yield and quality of crops such as vegetables, ornamentals, fibers, fruits, oil-seeds, plantations, medicinal, and aromatic plants (Olthof and Potter, 1972; Basu and Sukul, 1983; Haseeb, 1992, 1994; Singh and Haseeb, 1998).

On a worldwide basis, nematode may cause a quantitative reduction of 12% in the crop yield (Sasser, 1989). The losses in developing countries are relatively more than in the developed countries. About a hundred nematode diseases are of major economic significance in the world. Worldwide, the ten most important nematode genera are *Meloidogyne*, *Pratylenchus, Heterodera, Ditylenchus, Globodera, Tylenchulus, Xiphinema, Radopholus, Rotylenchulus* and *Helicotylenchus* (Veech and Dickson, 1987). Of the 70 described species of *Meloidogyne* causing root-knot disease in over six thousand plant species, about half a dozen of these species cause the greatest damage to vegetables, plantation crops, medicinal and aromatic plants. In fact they are responsible for 95% of total crop loss attributed to the nematodes (Dasgupta, 1997).

***Meloidogyne* spp.**

Root-knot nematodes, *Meloidogyne* species are considered the most potential nematode pests of many crops (Sasser, 1989) due to their worldwide distribution, extensive host range and highly advanced mode of parasitism. Root-knot nematodes induce development of characteristic galls on roots or other underground plant parts, in case of multiple infections on the nearby tissue, may coalesce to form large galls. The number, size and shape of the galls vary with the species and hosts. As a result of nematode induced cellular hyperplasia and hypertrophy, galls are formed on infected roots (Webster, 1972; Sasser, 1989).

The general appearance of the crop in a field is 'patchy' due to uneven distribution of the nematode. The poorly growing patches increase in size each year and may spread more rapidly in the direction of ploughing and irrigation water. The symptoms advance with the age of the crop and are more spectacular under drought or low fertility levels (Webster, 1972; Sitaramaiah, 1984; Sasser, 1989).

Several species of plant-parasitic nematodes have been reported to cause significant damage to mints. Among these *Meloidogyne* spp. and *Pratylenchus* spp. have been considered most important (Haseeb and Pandey, 1989; Haseeb, 1992, 1994; Haseeb and Shukla, 2000a, 2001a). Buhrer (1938) was the first to report the *M. arvensis* var. *piperascens* and *M. piperita* as the hosts of *Meloidogyne* sp.

After the introduction of mint cultivation in India, work regarding the nematode diseases of mints was initiated at CIMAP in 1983. For the first time in the world, root-knot disease of *M. arvensis* and *M. cardiaca* caused by *Meloidogyne incognita* (Kofoid and White) Chitwood and *Meloidogyne javanica* (Treub) Chitwood were reported in the experimental beds of CIMAP and in the tarai region of Uttar Pradesh (Anonymous, 1985, 1986, 1987; Haseeb and Pandey, 1989). Haseeb and Pandey (1989) also observed infection of both *Meloidogyne* species in the same root system, however, *M. incognita* dominated over *M. javanica*.

Phytopathogenic fungi

The fungi are a very large and diverse group of organisms, which have a unique life style. They have worldwide distribution and successfully exploit many different habitats (Isaac, 1992). They are responsible for a large number of diseases of human beings, animals and plants. Fungi affect mankind both directly and indirectly by destroying food, fabric and leather, and causing many diseases (Mehrotra, 1993).

The ability of fungi to adapt to a wide range of conditions makes the important group of pathogens. More than 10,000 species of fungi are responsible for causing diseases in plants. In general, all plants are attacked by some kinds of fungi and each of the parasitic fungi can attack one or many species of plants. Almost all plant pathogenic fungi spend part of their lives on their host plants and part in the soil or in plant debris on the soil (Agrios, 2000).

Various plant pathogenic fungi are known to infect mints, causing severe damage to the crop. Most species of fungi responsible for causing diseases of mint belong to the genera *Puccinia, Rhizoctonia, Macrophomina, Sclerotium, Fusarium, Verticillium, Curvularia, Alternaria, Erysiphae, Pernospora, Septoria* and *Sclerotinia* (Singh *et al.*, 1997; Singh and Haseeb, 1998).

***Sclerotinia* spp.**

The genus *Sclerotinia* belongs to Sclerotiniaceae, an important family of the class Ascomycotina. *Sclerotinia* spp. are polyphagous in nature, widespread and destructive pathogens of vegetables, ornamentals, field crops, medicinal and aromatic plants (Walker, 1969; Adams and Tate, 1975; Purdy, 1979; Tu, 1986; Agrios, 2000). The Sclerotinia diseases are known under a variety of names such as white mold, cottony rot, watery soft rot, stem rot, drop, crown rot, blosssom blight, pink joint among others (Chupp and Sherf, 1960; Walker, 1969; Purdy, 1979; Tu, 1986; Sattar *et al.*, 1995; Agrios, 2000).

The species of *Sclerotinia* can function either as soil-borne or airborne pathogens. Infections of above ground plant parts result from ascosporic inoculum, whereas soil-line infection may result either from ascospores or sclerotia. Below ground infection, however, results from mycelial germination of soil-borne sclerotia (Abawi and Grogan, 1979).

The distribution of various species of *Sclerotinia* is cosmopolitan, but they are most common in temperate regions (Riechert, 1958). They have been recognized for many years as serious pathogens of various vegetables, both in the field and during transit to market (Willetts and Wong, 1980). Partyka and Mai (1962) referred to a statement of Johnson (1902) that whole crops of

potatoes were destroyed by *Sclerotinia* diseases in Ireland and losses were more severe than those caused by late blight. Some reports, particularly from North America suggested that damage resulting from these fungal pathogens have increased significantly in recent years (Willets and Wong, 1980; Thompson *et al.*, 1984; Grau, 1988; Gulya *et al.*, 1989).

The symptoms caused by *Sclerotinia* vary somewhat with the host or affected plant part and with the environmental conditions. The most obvious and typical early symptom of *Sclerotinia* disease is the appearance on the infected plant of white fluffy mycelial growth which soon afterwards develops large, compact resting bodies or sclerotia (Agrios, 2000).

Stems of infected herbaceous plants first develop pale or dark brown lesions at their base. White cottony patches of fungal mycelium often quickly cover the lesions. In the early stages of lesion development in the stem, the foliage may show little sign of attack, and infected plants are easily overlooked until the fungus grows completely through the stem and the stem rots. Then, the foliage above the lesions wilts and dies more or less quickly (Purdy, 1979; Singh *et al.*, 1997; Agrios, 2000).

Sclerotinia sclerotiorum (Lib.) de Bary attacks over 360 species of plants in 54 families (Purdy, 1979). In 1973 report from Nebraska, 20% of the dry bean crop was lost to the ravage of *S. sclerotiorum* and damage was of $12 million (Lumsden *et al.*, 1975); similarly severe epidemics of Sclerotinia rot occurred on vegetable crops in Australia, causing serious economic losses (Letham *et al.*, 1976). In Ontario, a study placed the average incidence of white mold on bean crops at about 25% (Tu, 1986). The incidence of disease in different fields ranged from a trace to 100% (Tu, 1989).

In India, various crops viz. legumenous, vegetables, ornamental, medicinal and oil seed crops have reported to be severely damaged by *S. sclerotiorum* (Grewal and Pal, 1980; Singh, 1988; Dohroo *et al.*, 1990; Sattar and Alam, 1993; Sattar *et al.*, 1995; Paul, 1996; Singh *et al.*, 1997; Shivpuri and Gupta, 2001).

Interactions between root-knot nematodes and fungi

Nature does not work with pure cultures, thus in majority of cases, it is almost impossible to compartmentalize the individual effects of various microorganisms in the total disease syndrome. Therefore, the role of nematodes in plant disease problems can not be viewed in isolation from other microorganisms occupying the same habitat or ecological niche, since any significant disturbances in the biological equilibrium between the biotic components of the soil is bound to have an important bearing in the disease development, epidemiology of pathogens and finally in disease control. On account of the locomotion, habitat and feeding habits of the plant-parasitic nematodes, they are well suited for interactions with organisms, particularly soil-borne ones (Swarup, 1990).

Many microorganisms such as bacteria, fungi and viruses inhabit the rhizosphere along with nematodes. Nematodes interact with these microorganisms, especially fungi, resulting in an increase in losses to plants. Various workers have written several reviews on this subject (Miller, 1965; Pitcher, 1965; Powell, 1971; Khan and Muller, 1982; Haseeb, 1983; Sikora, 1984; Webster, 1985; Swarup, 1990; Luc *et al.,* 1993; Khan, 1993; Back *et al.*, 2002). The increase in losses due to nematodes and fungi interacting can be either additive or synergistic, with both causing disease complexes (Back *et al.*, 2002).

As early as 1892, Atkinson recognized the ability of nematodes to interact with fungi when he observed that infection by root-knot nematodes increased the severity of Fusarium wilt in cotton. Since this first report, interest has been generated to study the role of plant-parasitic nematodes particularly root-knot nematode in fungal disease complexes on number of crops (Powell, 1963; Porter and Powell, 1967; Powell *et al.*, 1971; Garcia and Mitchell, 1975; Golden and Van Gundy, 1975; Griffin, 1986; MacGuidwin and Rouse, 1990; Walker *et al.*, 1999; Perveen *et al.*, 1999a, b; Anwar and Khan, 2002). Very little information is available regarding the interaction of root-knot nematodes with other soil-borne pathogens particularly fungi on mint. Haseeb and Shukla (2000b) reported

that the severity of root-knot disease of Japanese mint was increased under field conditions in the presence of several soil-borne fungi, particularly species of *Fusarium* and *Rhizoctonia*.

A literature review revealed that a number of workers have studied the effect of root-knot nematodes on the growth and yield of various crops (Webster, 1972; Sitaramaiah, 1984; Haseeb and Butool, 1989; Sasser, 1989; Haseeb and Pandey, 1989; Luc *et al.*, 1993; Haseeb and Shukla, 2000a, 2001b); the effects of *Sclerotinia* on several crops (Abawi and Grogan, 1975; Purdy, 1979; Tu, 1987; Grau, 1988; Sala *et al.*, 1996) and on disease complexes of root-knot nematodes and soil-borne fungi (Powell, 1971; Garcia and Mitchell, 1975; Golden and Van Gundy, 1975; Griffin, 1986; MacGuidwin and Rouse, 1990; Khan, 1993; Walker *et al.*, 1999; Back *et al.*, 2002). However, studies regarding the quantification of damage due to the infection of *S. sclerotiorum* on *M. arvensis* have not been conducted. Similarly, studies regarding the singular or combined effect of *M. incognita* and *S. sclerotiorum* on growth and oil yield of Japanese mint and factors affecting on the severity of disease and measures to manage the losses due to these pathogens are not yet been investigated.

Due to the importance of this crop, an ever-increasing demand for menthol in cosmetic and pharmaceutical industries, and the damaging potential of root-knot nematodes alone and in combination with several soil-borne fungi, the following studies were undertaken.

(i) Survey for the association of plant-parasitic nematodes and pathogenic soil-borne fungi on *M. arvensis*.

(ii) Determine the effect of different initial inoculum levels of *M. incognita* on root-knot disease development, nematode multiplication, growth, oil yield and biochemical changes in plants of *M. arvensis* cv. Gomti.

(iii) Study the effect of different initial inoculum levels of *S. sclerotiorum* on disease development, growth, oil yield and biochemical changes in plants of *M. arvensis* cv. Gomti.

(iv) Investigate the effect of *M. incognita* and *S. sclerotiorum* alone or in combined inoculations on disease development, nematode multiplication, growth, oil yield and biochemical changes in plants of *M. arvensis* cv. Gomti.

(v) Study the effect of different pH levels on disease development, nematode multiplication, growth, oil yield and biochemical changes in *M. incognita* and/or *S. sclerotiorum* inoculated plants of *M. arvensis* cv. Gomti.

(vi) Determine the effect of different soil types on disease development, nematode multiplication, growth, oil yield and biochemical changes in *M. incognita* and/or *S. sclerotiorum* inoculated plants of *M. arvensis* cv. Gomti.

(vii) Study the comparative efficacy of pesticides, neem seed powder and bio-control agents on root-knot disease development, nematode multiplication, growth and oil yield in *M. incognita* inoculated plants of *M. arvensis* cv. Gomti.

(viii) Determine the comparative efficacy of pesticides, neem seed powder and bio-control agents on disease development, growth and oil yield in *S. sclerotiorum* inoculated plants of *M. arvensis* cv. Gomti.

(ix) Investigate the comparative efficacy of pesticides, neem seed powder and bio-control agents on disease development, nematode multiplication, growth and oil yield in *M. incognita* and *S. sclerotiorum* inoculated plants of *M. arvensis* cv. Gomti.

(x) Study the comparative efficacy of pesticides, neem seed powder and bio-control agents on disease development, nematode multiplication, growth and oil yield in plants of *M. arvensis* cv. Gomti grown in a field naturally infested with *M. incognita* and *S. sclerotiorum*.

Chapter 2

Survey

1. Survey for the association of plant-parasitic nematodes and pathogenic soil-borne fungi on *M. arvensis*

The terms SURVEY is derived from two Latin words 'Sur' and 'Video' means to over and see respectively i.e. a general survey or inspection or collection of data for mapping. Surveillance denotes repeated or sequential survey of the same place or locality for taking observations to see the changes or fluctuations in the object of study. The type of survey may be qualitative- involving the identification of different species present in that area or quantitative- involving the estimation of population of one or more species present in that area. The survey may be extensive- when it is covering a vast area for study or intensive- when more accurate detailed knowledge is required. Survey may be locality-wise, host wise or pathogen-wise according to the requirement.

REVIEW OF LITERATURE

Plant-parasitic nematodes

Plant-parasitic nematodes affect the production and economy of the crops in diverse ways such as (i) reduction in quality and quantity of crops, (ii) need of additional fertilizer, water and application of nematicides, and (iii) impediment of production and trade by phytosanitory regulations (Weicher, 1967).

A worldwide survey covering 75 countries involving 371 nematologists revealed that *Meloidogyne, Pratylenchus, Heterodera, Ditylenchus, Globodera, Tylenchulus, Xiphinema, Radopholus, Rotylenchulus* and *Helicotylenchus* are the most damaging genera of plant-parasitic nematodes occurring in these countries (Sasser and Freckman, 1987).

On a global scale, root-knot nematodes (*Meloidogyne* spp.) are the most destructive of all the nematode parasites of agricultural and horticultural crops. They are most prevalent in the tropics and sub-tropics, where they cause damage to almost all types of plants like vegetables, legumes, cereals, horticultural, plantation, medicinal and aromatic plants (Webster, 1972; Haseeb, 1992, 1994; Luc *et al.,* 1993; Sitaramaiah, 1994; Haseeb and Shukla, 2000a, 2001a, 2002).

As early as 1855, Berkeley for the first time reported root-knot nematode disease of glass house cucumber from England, he named the nematode as 'vibrios' and the disease as 'excrescence'.

Buhrer (1938) for the first time reported *M. arvensis* var. *piperascens* and *M. piperita* as the hosts of *Meloidogyne* sp., whereas, Horner and Jensen (1954) noticed a very high population of *Meloidogyne hapla* in the rhizosphere of several species of mints in Western Oregon.

Skotland and Menzies (1957) reported *M. hapla* as the most prevalent nematode species associated with *M. piperita* cv. Mitcham, *M. cardiaca* cv. Scotch and *M. spicata* cv. Native in the Yakima valley and the Columbia basin.

Haseeb *et al.* (1980, 1982, 1984, 1985) and Haseeb and Khan (1983) reported several medicinal, aromatic and weed plants for the first time as the hosts of *M. incognita* and *M. javanica.* O' Bannon *et al.* (1982) reported *M. spicata, M. piperita* and *M. cardiaca* as the good hosts of *Meloidogyne chitwoodi* in the area of Pacific North West.

Maqbool *et al.* (1985) reported *M. piperita* as a host of *Meloidogyne* spp. from Pakistan. They observed severe galling on the roots of *M. piperita* due to the infestation of *M. hapla.*

For the first time in India, root-knot disease of *M. arvensis* and *M. cardiaca* caused by *M. incognita* and *M. javanica* were noticed in the experimental beds of CIMAP, Lucknow and in the Tarai region of Uttar Pradesh (Anonymous, 1985, 1986, 1987).

Haseeb and Pandey (1989) conducted a survey of *M. arvensis* at experimental beds of CIMAP, Lucknow and the Tarai region of Uttar Pradesh. They reported the infestation of *M. incognita* and *M. javanica* in the same root system. However, *M. incognita* dominated over *M. javanica.*

Khan and Reddy (1991) conducted surveys during 1987-88 around Bangalore and Tarai region of Uttar Pradesh. They found that *M. piperita* roots and soil harbour *M. incognita, R. reniformis* and *Pratylenchus brachyurus* in Bangalore and *M. incognita* and *P. zeae* in Tarai region.

Pandey *et al.* (1992) reported *M. arvensis* cv. MAS-1 as a most susceptible host of *M. incognita.* Haseeb (1992, 1994) reported that the different cultivars of *M. citrata, M. piperita* and *M. spicata* as hosts of *M. incognita* and *M. javanica.*

Shukla (1997, 1998) carried out a comprehensive survey of mint growing areas of Uttar Pradesh, and reported the presence of important plant-parasitic nematodes in the rhizosphere of *M. arvensis, M. piperita* and *M. spicata. Meloidogyne* species was most dominant nematode followed by *Helicotylenchus, Tylenchorhynchus, Pratylenchus, Hoplolaimus, Rotylenchulus* and *Xiphinema* respectively.

Sivkumar and Vadivellu (1997) studied the association of plant-parasitic nematode with various medicinal and aromatic plants in the Nilgiris. They reported that *M. hapla, M. incognita, Helicotylencus incisus* and *Pratylenchus coffeae* were found to be consistently associated with *M. arvensis, M. citrata* and *M. piperita* plants.

Phytopathogenic fungi

Fungi are one of the major groups of pathogens, which cause severe damage to crops (Agrios, 2000). The great potential of fungi to adopt to a wide range of conditions makes it an important group of pathogens (Swarup, 1990). It is well documented in the literature that the plant pathogens, either soil borne or foliage pathogens inflict considerable economic losses to various crops.

As early as 1950, in USA, Nelson reported wilt/rot of peppermint due to *Verticillium dahliae, V. albo-atrum, V. albo-atrum* var. *menthae* and *V. nigrescens,* whereas, wilt of Bergamot mint was due to *V. albo-atrum.* The pathogens were found to be responsible for reduction in yield of herb and essential oil, which resulted in abandoning the cultivation of thousands of acres of mint in U.S.A.

Steenland and Bruke (1950) noticed root rot of peppermint due to *Typhula itoana* in Grand Island, Oregon, while Sharma and Mahmud (1951) isolated *Rhizoctonia solani* from spearmint exhibiting symptoms in Nagpur.

In Italy, Peasante (1955) found peppermint wilt was caused by *V. albo-atrum* var. *menthae.* Skotland and Menzies (1957) observed a considerable damage in peppermint due to *Sclerotium sclerotiorum* in Washington.

Ganguli and Pandotra (1962) reported the occurrence of *R. solani* on Japanese mint from Jammu and Kashmir. Later on, Husain and Janardhanan (1965) also identified *R. solani* (*Macrophomina phaseolina*) causing stolon rot of mint in Jammu.

In a study, Pandotra and Sastry (1968) observed root and collar rot of Japanese mint due to *Sclerotium rolfsii* in adjoining areas of Jammu. They further stated that the pathogen formed mycelial strands followed by sclerotia formation around the stem.

Paizs and Nagy (1975) observed the infection of *Phoma strasseri* on *M. piperita* and *Mentha sacholinensis* from Hungary. The pathogen caused discoloration of leaves followed by wilting. Layton (1976) noticed collar rot symptoms on *M. arvensis* var. *piperascens* from Papua New Guinea due to *Marasmiellus epochnous.* Beside the above symptoms, the infected plants also showed wilting followed by death of the shoots.

Sattar and Husain (1976) reported the stolon and root-rot disease of *M. arvensis* sub. sp. *haplocalyx* var. *piperascens* caused by *Thielavia* (*Thielaviopsis*) *basicola.*

Sharma and Munjal (1978) observed the symptoms of blight on *Mentha* sp. due to *R. solani* in Himachal Pradesh. Severe wilt disease of *M. arvensis* sub. sp. *haplocalyx* var. *piperascens* caused by *Fusarium oxysporum* was reported from different parts of India (Sattar and Husain, 1978).

Singh and Singh (1991) observed severe stolon rot of *M. arvensis* due to *R. solani* and *M. phaseolina* in Bangalore. Gaetan and Gally (1993) reported anthracnose of peppermint due to *Colletotrichum gloesporiodes* (*Glomerella cingulata*) in Argentina.

Trueman *et al.* (1995) reported from U.S.A. the vascular wilt disease caused by *F. oxysporum* on several species of herbs, including *M. arvensis* sub. sp. *haplocalyx* var. *piperascens.*

During a systematic survey of fungal diseases of menthol mint, Singh *et al.* (1997) observed stolon decay disease, due to *S. sclerotiorum* on *M. arvensis* var. *piperescens.* Shukla *et al.* (2001) during survey of commercial cultivation of menthol mint in the surrounding districts of Lucknow and Tarai region of Uttar Pradesh observed new stem blackening and rot disease, due to *Botryodiploidia theobromae.*

MATERIALS AND METHODS

A comprehensive survey of Aligarh, Bulandshahar, Badaun, Barielly, Etah, Moradabad and Rampur districts located in the Western and Tarai region of Uttar Pradesh was carried out in the month of March 2000, at the initial growth period of crop and during the month of May 2000, when the crop was fully grown by taking soil and root/sucker samples from the rhizosphere of apparently diseased plants of *M. arvensis* to identify the prevailing species of plant-parasitic nematodes, particularly root-knot nematode, and soil borne fungi.

1.1 Symptomatology

The above and belowground symptoms produced due to infection of nematodes and fungi to Japanese mint in fields were observed and details of symptoms were recorded.

1.1 Collection of soil and root/sucker samples

1.2.1 Soil samples

Soil samples were collected with the help of auger (khurpi) from the rhizosphere of *M. arvensis.* From each field, 5-20 sub samples were taken randomly according to the area of the field. The sub-samples were mixed thoroughly and approximately 500 g soil was placed into polythene bags and tagged with relevant information. The samples were brought to the laboratory and kept in refrigerator until plant-parasitic nematodes were extracted.

1.2.2 Root/sucker samples

Root/sucker samples were also collected and tagged simultaneously from apparently diseased plants of *M. arvensis,* in the same manner as described for soil samples. These samples were also kept in refrigerator until the root-knot index and percent root infection were graded, and isolation of fungi and nematodes was done from roots and suckers.

1.3 Grading of root-knot index

The root/sucker samples of *M. arvensis* collected from different localities were washed thoroughly under running tap water to remove the adhering soil particles and afterwards root-knot index was graded on a scale of 0-4 (Taylor and Sasser, 1978), where:

0	=	No galling	(0%)
1	=	Light galling	(1% - 25%)
2	=	Moderate galling	(26% - 50%)
3	=	Heavy galling	(51% - 75%)
4	=	Severe galling	(76% - 100%)

1.4 Determination of percent roots/suckers infection by fungi

The washed suckers and roots were cut into pieces of 1.0 cm length and treated with 10% KOH solution and were kept at 90°C in a hot air oven for one hour. The root/sucker segments were washed again with distilled water, acidified and were stained with trypan blue (0.05% in lactophenol) as described by Phillips and Haymen (1970). Ten stained root pieces were mounted on a slide in lactophenol and observed under a microscope. Ten longitudinal sections of suckers were also mounted on a separate slide in the same manner. The portion of length of sucker and root segments, which showed the presence of hyphae of fungi, was estimated. The percent infection was calculated by measuring the infected portion in relation to total length of sucker and root pieces (Biermann and Lindermann, 1981).

1.5 Isolation and identification of plant-parasitic nematodes and fungi

1.51 Isolation of nematodes from soil

Nematodes were isolated from soil using Cobb's sieving and decanting technique in conjunction with Baermann funnel (Southey, 1986). A 250 g soil sample was placed into a plastic bucket, and the bucket was filled with ten liters of water. The suspension was stirred gently and thoroughly to break the soil aggregates and release nematodes into water. The suspension was allowed to settle for 2 minutes for heavy soil particles to sink to the bottom of the bucket. The suspension was decanted through sieves with the pore openings 710, 63, 45, 39 μm. The entire process was repeated twice for improved nematode recovery. Thereafter, the nematodes and remaining debris were collected in a beaker by gently rinsing from the sieve.

To obtain a clean suspension of the nematodes, the Baermann's funnel method was employed. A double folded fine tissue paper was placed on sieves with openings of 710 μm in Baermann funnels. The funnel was placed on a support and water added. The suspension containing nematodes was poured over the tissue paper. The nematodes being active made their way through the tissue paper into the water. After 48 hours the nematodes were recovered by opening the clip of the rubber tube connected to the bottom of the Baermann funnel, thus allowing the nematodes in water to flow out in a beaker and final volume of the suspension was made to 100 ml by adding tap water.

For counting the plant-parasitic nematodes, one ml nematode suspension was poured into a shallow multichambered counting dish and observed under the stereoscopic microscope (Doncaster, 1962). The counting of each sample was done twice to reduce the error. From the resulting nematode count, nematode population in 100 ml suspension i.e. 250 g soil was calculated.

1.5.2 Isolation of nematodes from roots/suckers

Isolation of nematodes from roots/suckers was done by mechanical maceration. Thoroughly washed five gram roots and suckers from each sample

were cut into small pieces and were macerated in an electric warring blender with sufficient amount of water. The suspension was cleared by sieving through a 710-μm openings sieve and the final volume of the suspension was made up to 100 ml by adding tap water (Southey, 1986).

For counting the nematodes, one ml nematode suspension was poured into a shallow multichambered counting dish and observed under the stereoscopic microscope (Doncaster, 1962). The counting of each sample was done twice to reduce the error. From the resulting nematode count, nematode population in 100 ml suspension i.e. 5 g roots/suckers was calculated.

1.5.3 Identification of root–knot nematode species from roots/suckers

Ten fully mature female specimens of the nematodes were excised from the galled tissues of the roots/suckers of *M. arvensis,* collected during survey from each and every locality. Perineal patterns from females were cut and stained in hot acid fuschin (0.01%) and mounted in lactophenol (Taylor and Netscher, 1974). Specific identification of the nematodes was done in the laboratory by the close examination of perineal pattern under a stereoscopic microscope (Southey, 1986).

Identification of plant-parasitic nematodes

The identification was done with the help of literature available. The following literature was used for identification of nematodes.

(i) Various national and international journals of repute.

(ii) Description of Plant-Parasitic Nematodes, issued by Commonwealth Institute of Helminthology, England.

(iii) Pictorial Key to Genera of Plant-Parasitic Nematodes, 1975. By, W.F. Mai and H.H. Tyon, Constok publishing associates Ithaca and London.

(iv) Plant Nematology, 1982. Edited by J.F Southey, A.D.A.S. Plant Pathology laboratory, Harpenden, U.K.

(v) Tylenchida Parasites of Plants and Insects, 2000. By M.R. Siddiqui, published by Commonwealth Agricultural Bureau's, U.K.

(vi) Introduction to Research on Plant Nematology, 1971. An FAO Guide to the study and control of plant-parasitic nematodes. By Albert, L. Taylor. FAO of the United Nations.

1.5.4 Isolation and identification of fungi from roots and suckers of *M. arvensis*

Apparently infected portion of aerial as well as roots/suckers of *M. arvensis* plants collected during survey were surface sterilized in 0.1% sodium hypochlorite solution and were placed in petri plates containing potato dextrose agar (PDA) medium (Riker and Riker, 1936). Petri plates were incubated in a BOD incubator at 27 ± 1°C for the growth of the fungus. Three days after inoculation, fungal colonies were transferred separately to culture tubes containing PDA medium and were kept for a week in an incubator at 27 ± 1°C. The pure cultures obtained after incubation were replicated and were stored in the refrigerator. Slides were prepared from various fungal cultures and were observed under a microscope. On the basis of cultural characters and microscopic observations, fungi were identified. For further confirmation of fungi, the fungal cultures were sent to Indian Type Culture Collection (ITCC), Plant Pathology Division, Indian Agricultural Research Institute (IARI), New Delhi, India.

RESULTS

The observations, regarding the visual symptoms, nematode population in soil and roots/suckers, root-knot index, identification of nematodes and fungi associated with *M. arvensis*, percent roots/suckers infection by fungi recorded during the survey are presented below.

1.1 Symptomatology

Visual observations of fields in March during Survey I showed no specific symptoms of nematode attack. In some localities, cup-shaped brown coloured apothecia of *Sclerotinia sclerotiorum* were observed on the soil surface near the plants (Plate 1A and 2). Observations made on the roots and suckers indicated the beginning of galling caused by root-knot nematode and at this

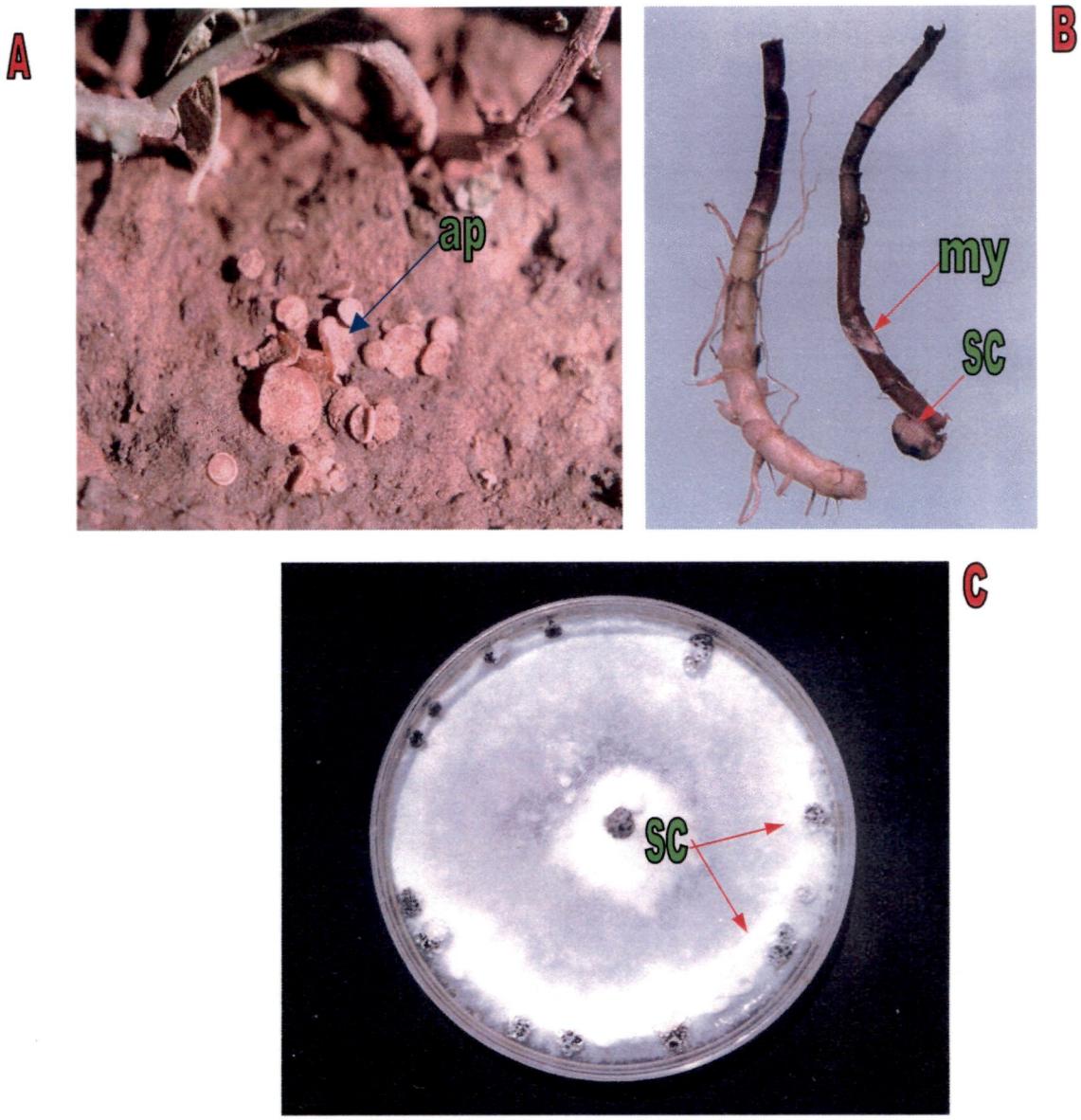


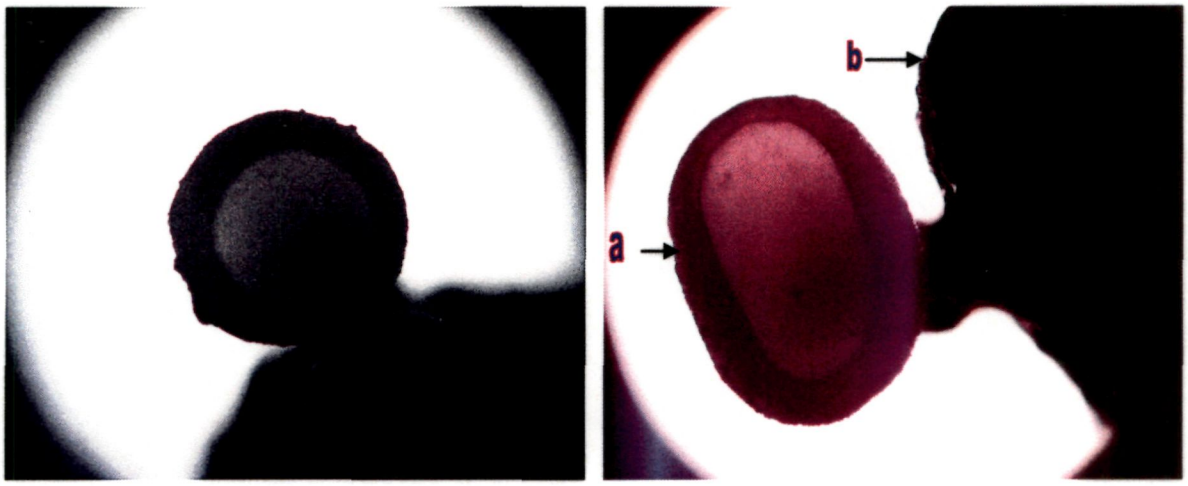
Plate 1: ***Sclerotinia sclerotiorum.***

A: Apothecia of *S. sclerotiorum* in the rhizosphere of *M. arvensis* cv. Gomti.

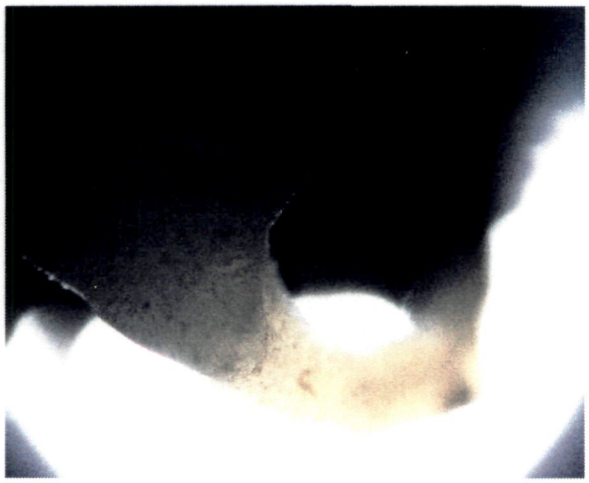
B: Healthy (left) and infected sucker of *M. arvensis* cv. Gomti, showing growth of mycelia and sclerotium of *S. sclerotiorum.*

C: Culture, *S. sclerotiorum* on potato dextrose agar.

ap = apothecia my = mycelium sc = sclerotia



Sclerotium bearing apothecium
a- Apothecium, b - Sclerotium



Attachment of apothecium

Side view of apothecium

Plate 2: Apothecia Formation

stage, egg masses were rarely observed. In several samples, water soaked areas on suckers caused due to the infection of *S. sclerotiorum* were also observed.

In May during Survey II, the crop was fully grown and the symptoms of patchy stunting were commonly observed. Leaves of severely stunted plants were mostly observed to be yellowish in colour and smaller in size. Wilting of plants was also observed in many fields (Plate 3). The roots and suckers of stunted/wilted plants had very severe galling caused by root-knot nematodes. Shiny egg masses were observed on the galls formed on the roots and suckers of surviving plants. Several roots/suckers were also dark brown to black in colour and showed rot (Plate 4). In the presence of good moisture in soil, decayed roots and suckers with white fluffy mycelium were also observed. At various locations black coloured sclerotia of *S. sclerotiorum* were also found attached to the infected suckers (Plate 1B).

1.2 Plant-parasitic nematodes in soil

Nematode population in soil indicated that the total populations varied from 100-2000 nematodes/250 g of soil during the second survey (Table 1 and 2). The plant-parasitic nematodes isolated and identified from the soil were second stage juveniles of *Meloidogyne* spp., and different stages of *Tylenchorynchus brassicae, T. vulgaris, Rotylenchulus reniformis, Pratylenchus thornei, Helicotylenchus indicus* and *Hoplolaimus indicus.* Genera not identified to species included *Tylenchus*, *Xiphinema*, *Longidorous* and *Criconimoides*.

In general, *Meloidogyne* spp. J2 was the dominant population in the rhizosphere of *M. arvensis.* However, in many samples either *Tylenchorhynchus* spp., *R. reniformis* or *P. thornei* dominated the population. Among other plant-parasitic nematodes, *Hel. indicus* and *Hop. indicus* were found consistently in higher numbers, while *Tylenchus* sp., *Xiphinema* sp., *Longidorous* sp. and *Criconimoides* sp. were found occasionally.

1.2.1 Average percent occurrence of nematodes

Data regarding percent occurrence (total number of sample basis) of different genera of plant-parasitic nematode in the area under study indicated

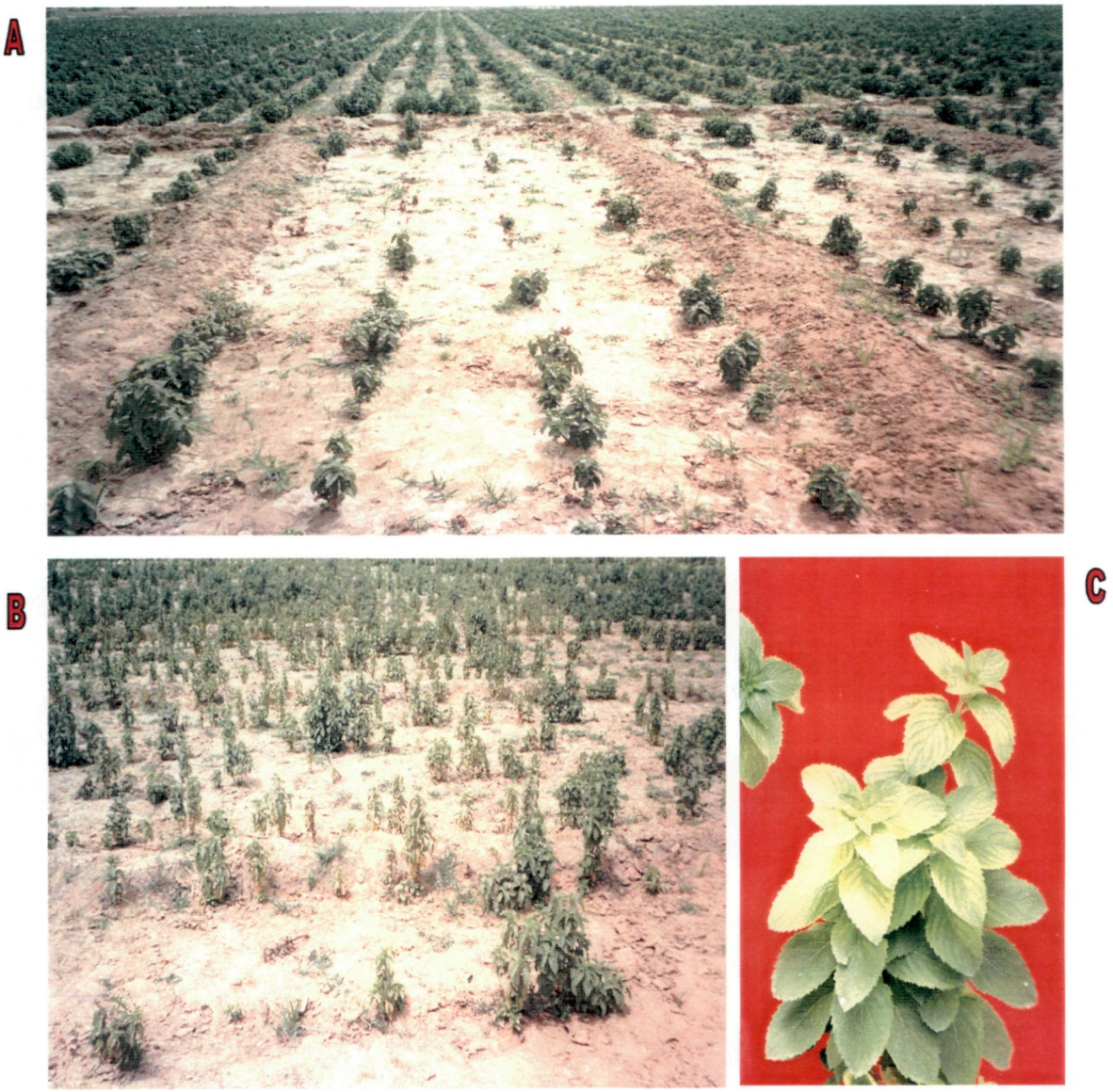


Plate 3: Symptoms on *Mentha arvensis* in farmer's field.

A: Showing wilt, stunting with patchy appearance of the crop.

B: Showing wilting, yellowing, stunting and patchy appearance of the crop.

C: A view of *M. arvensis* plant showing yellowing of leaves.

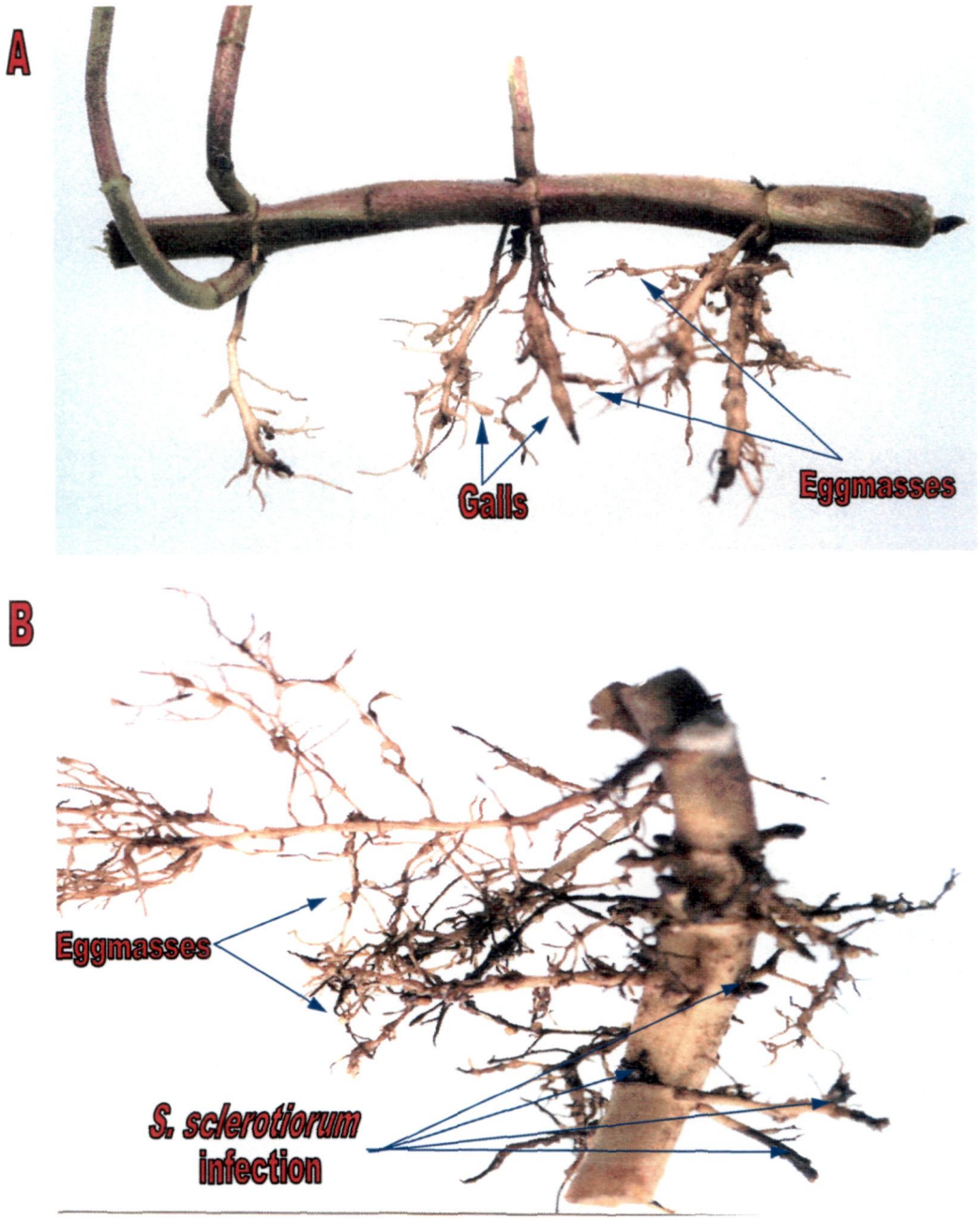


Plate 4: Symptoms on roots and suckers of *M. arvensis*.

A: Showing galls and egg masses of *M. incognita*.

B: Showing egg masses of *M. incognita* and *S. sclerotiorum* infection on roots.

Table 1: Initial survey of plant-parasitic nematodes and soil borne fungi associated with *Mentha arvensis*, March 2000.

Districts/Localities	Nematode population/ 250 g soil	*Meloidogyne* spp./ g roots & suckers	Percent occurrence							[b]Root-knot index	[c]Roots & suckers infection
			Meloidogyne spp.	*Hel. indicus*	*Hop. indicus*	*P. thornei*	*R. reniformis*	*Tylenchorhynchus* spp.	Others[a]		
Rampur											
Badhaiya	200	20	11.1	12.2	13.7	11.1	-	29.7	22.2	0.15	35
Bagi	100	60	20.0	-	5.0	25.0	-	25.0	20.0	0.30	25
Bilaspur	600	40	10.0	8.0	-	35.0	-	25.0	22.0	0.25	15
Hasanpur	300	40	22.5	18.0	10.5	-	10.0	16.0	23.5	0.20	20
Jiwaijajit	200	60	20.0	-	10.0	8.0	-	35.0	27.0	0.25	30
Kemari	100	40	12.5	22.0	10.5	8.5	-	32.0	14.5	0.18	10
Mansoorpur	300	20	10.0	25.0	20.5	5.0	10.0	12.5	17.5	0.15	20
Milak	300	60	17.5	22.5	5.0	12.5	-	12.5	30.5	0.30	18
Mullakhera	200	20	25.0	20.0	7.5	4.5	5.0	20.0	35.0	0.10	15
Ram Nagaria	1200	100	25.0	-	8.0	10.0	-	20.0	37.0	0.50	22
Simara	100	20	25.0	7.5	5.0	32.0	-	-	30.5	0.10	25
Talakpur	700	60	12.5	8.5	7.0	5.0	-	35.0	32.0	0.25	10
Usufpur	600	60	15.0	22.0	-	12.0	10.0	25.0	16.0	0.30	15
Vilaspur	200	40	10.0	18.0	5.5	13.0	-	25.0	28.5	0.20	20
Visharad Nagar	800	100	35.0	-	25.0	12.5	-	-	12.5	0.60	30
Bareilly											
Amla	400	20	7.5	55.0	7.5	-	-	24.5	5.0	0.10	15
Attamanda	1400	100	18.0	-	-	12.0	47.0	13.0	10.0	0.60	20
Bilva	600	40	15.5	-	35.0	-	25.0	15.0	9.5	0.20	22
Bhamora	1800	100	8.5	15.0	10.0	20.0	20.0	12.5	13.5	0.60	18
Damora	600	40	10.0	-	-	15.0	20.0	45.0	10.0	0.15	25
Darka	200	20	20.0	20.0	-	25.0	-	20.0	15.0	0.10	30

Table 1 continued ... ...

Devi	200	40	50.0	-	25.0	-	25.0	-	-	0.20	10
Devehara	700	80	20.0	-	-	-	50.0	25.0	5.0	0.30	20
Dev Rania	400	20	15.0	20.0	15.0	25.0	-	25.0	-	0.10	10
Dhaneta	200	80	20.0	35.0	-	-	30.0	-	15.0	0.35	35
Jadavpur	2000	80	6.0	21.0	7.5	-	48.0	15.0	2.5	0.30	30
Kasumra	200	80	50.0	10.0	12.0	-	-	8.0	20.0	0.35	25
Kulchha	1200	80	10.0	24.0	-	-	63.0	-	3.0	0.25	25
Mirganj	1000	100	30.0	20.0	-	-	-	20.0	30.0	0.65	20
Mohanpur	300	-	2.5	-	15.0	25.0	-	48.0	9.5	-	15
Moradabad											
Bhikanpur	700	140	28.0	22.0	10.5	9.5	-	15.0	15.0	0.85	2.5
Dalpatpur	400	100	20.0	-	7.0	15.0	-	40.0	18.0	0.70	20
Faridpur	800	180	50.0	25.0	-	-	-	25.0	-	1.00	40
Firozpur	800	120	18.5	-	10.0	15.0	20.0	25.0	10.0	0.75	35
Jahid Nagar	700	100	50.0	-	15.0	-	-	30.0	5.0	0.70	45
Jatpura	1200	120	15.0	15.5	8.0	7.0	-	40.0	7.0	0.80	24
Ladpura	800	140	23.5	20.0	21.5	-	-	25.0	10.0	0.90	32
Mainathai	600	80	35.0	-	5.0	11.5	28.0	12.0	8.5	0.30	28
Maulagarh	700	80	25.0	15.0	20.0	15.5	-	15.0	9.5	0.25	28
Munda Pandey	1000	120	28.0	30.0	-	27.0	-	15.0	10.0	0.75	25
Nasilpur	700	80	20.0	28.0	5.5	8.0	32.0	-	6.5	0.20	22
Orchhi	700	140	40.0	15.5	5.0	8.5	-	25.0	16.0	0.90	35
Sambhal	1000	160	35.0	-	10.0	-	38.0	5.0	12.0	1.00	38
Sahibabad	600	100	38.0	20.0	25.0	-	-	12.0	5.0	0.55	28
Sirsi	900	100	40.0	-	5.0	-	55.0	-	-	0.60	50
Badaun											
Bisauli	1000	80	18.5	15.0	7.5	-	10.0	35.0	14.0	0.25	35
Chhaduiya	600	-	-	5.0	5.0	-	48.0	10.0	32.0	-	15
Chaitrapur Madiya	700	40	15.0	42.5	5.0	-	-	7.5	30.0	0.15	30
Dharampal Jirauli	700	60	18.5	-	-	25.0	-	40.0	17.0	0.20	20

Table 1 continued ... ...

Dharampur	600	80	35.0	12.5	5.0	-	37.5	-	10.0	0.30	18
Etauwa	1200	80	18.0	5.0	-	15.0	-	27.5	34.5	0.35	20
Faizganj	1000	100	30.0	35.0	-	-	25.0	-	10.0	0.60	40
Hasanpur	600	100	42.0	-	43.0	-	-	15.0	-	0.65	35
Hatsa	500	80	25.0	10.0	5.0	-	40.0	-	20.0	0.35	20
Jaraga	1200	80	10.0	-	-	-	70.0	-	20.0	0.30	15
Mundia	400	20	18.0	7.0	5.0	10.0	35.0	-	24.0	0.10	20
Noorpur	700	40	12.5	-	40.0	10.0	-	10.0	27.5	0.15	15
Sikrapur	200	40	30.0	-	-	-	50.0	-	20.0	0.20	22
Waheedpur	200	20	20.0	10.0	15.0	-	25.0	15.0	15.0	0.10	25
Lelai	1200	100	22.0	20.0	20.5	10.0	-	-	27.5	0.55	28
Bulandshahar											
Badarpur Nagla	500	80	20.0	20.0	25.0	-	10.0	-	25.0	0.35	35
Chhatari	300	80	35.0	-	12.5	-	10.0	8.0	10.0	0.40	30
Jirauli	600	100	20.0	-	5.0	15.0	-	17.5	10.0	0.60	25
Nibari Bangar	300	80	33.0	7.5	7.5	10.0	10.0	7.5	25.0	0.35	25
Simrauli	400	40	25.0	7.5	10.0	15.0	-	25.0	17.5	0.15	30
Udaypur Khurd	400	60	27.5	15.0	18.5	7.5	-	7.5	24.0	0.25	35
Aligarh											
Jargawan	200	40	20.0	10.0	-	20.0	-	25.0	25.0	0.20	35
Jirauli Dhoomsingh	500	80	20.0	12.5	-	17.5	25.0	20.0	5.0	0.35	28
Malaypur	300	40	17.5	10.0	5.0	-	10.0	30.0	27.5	0.20	20
Raipur Dalpat pur	200	80	50.0	-	25.0	-	-	25.0	-	0.40	24
Etah											
Allah Nagar	200	-	2..5	25.0	20.0	18.5	-	34.0	-	-	15

[a]Others: - *Tylenchus* sp., *Xiphinema* sp., *Longidorous* sp., *Criconimoides* sp.
[b]Root-knot index: 0 = 0%, 1 = 1-25%, 2 = 26-50%, 3 = 51-75%, 4 = 76-100%.
[c]Percent roots and suckers infection by fungi.

Table 2: Survey of plant-parasitic nematodes and soil-borne fungi associated with *Mentha arvensis,* May 2000.

Districts/Localities	Nematode population/ 250 g soil	*Meloidogyne* spp./ g roots & suckers	Percent occurrence							[b]Root-knot index	[c]Roots & suckers infection
			Meloidogyne spp.	*Hel. indicus*	*Hop. indicus*	*P. thornei*	*R. reniformis*	*Tylenchorhynchus* spp.	Others[a]		
Rampur											
Badhaiya	900	240	32.0	9.0	10.0	10.0	-	20.0	20.0	1.00	55
Bagi	200	80	40.0	-	5.0	20.0	-	20.0	15.0	0.65	40
Bilaspur	1500	60	10.5	7.5	-	35.0	-	25.0	22.0	0.50	30
Hasanpur	400	120	35.0	13.0	7.5	-	5.0	16.0	23.5	0.75	35
Jiwaijajit	600	180	25.0	-	5.5	7.5	-	35.0	27.0	0.85	50
Kemari	100	40	25.5	20.0	10.0	8.0	-	22.0	14.5	0.25	25
Mansoorpur	800	260	25.0	17.5	17.5	5.0	7.5	10.0	17.5	1.00	35
Milak	600	100	25.0	20.0	5.5	10.0	-	10.5	29.5	0.75	35
Mullakhera	500	80	39.0	15.0	7.5	5.5	5.0	15.0	13.0	0.65	40
Ram Nagaria	2000	220	25.0	-	10.0	10.0	-	20.0	35.0	0.75	35
Simara	400	260	40.0	7.5	5.0	25.0	-	7.5	15.0	1.00	45
Talakpur	1700	280	30.0	7.5	5.0	5.0	-	37.0	15.5	0.65	30
Usufpur	1000	120	22.0	15.0	-	10.0	10.0	25.0	18.0	0.75	30
Vilaspur	200	60	20.5	20.0	3.3	13.3	-	30.0	20.0	0.35	45
Visharad Nagar	1800	240	40.0	-	20.0	10.0	-	-	15.0	1.00	55
Bareilly											
Amla	1100	400	47.5	20.0	5.5	-	-	14.5	12.5	1.40	35
Attamanda	3600	460	30.0	5.0	-	10.0	35.0	15.0	5.0	1.75	40
Bilva	1800	300	30.0	-	20.0	-	25.0	15.0	10.0	1.10	45
Bhamora	3200	240	15.0	15.0	10.0	18.5	20.0	15.0	6.5	0.90	34
Damora	1500	280	25.0	-	-	7.0	18.0	50.0	-	1.00	48

Table 2 continued ... ...

Darka	800	80	25.0	20.0	-	25.0	-	20.0	10.0	0.50	55
Devi	200	40	75.0	-	25.0	25.0	-	-	-	0.25	30
Devehara	1700	320	30.0	-	-	-	50.0	20.0	-	1.00	40
Dev Rania	4200	400	35.0	15.0	15.0	25.0	-	20.0	-	1.40	30
Dhaneta	800	180	40.0	30.0	-	-	25.0	-	5.0	0.85	50
Jadavpur	4100	320	17.5	9.5	7.5	-	45.0	18.0	2.5	1.25	50
Kasumra	800	180	55.0	5.0	12.5	-	10.0	7.5	10.0	0.85	45
Kulchha	2600	300	12.5	25.0	-	-	60.0	-	2.5	1.00	48
Mirganj	2000	400	40.0	20.0	-	-	23.0	15.0	2.0	1.50	40
Mohanpur	800	-	2.5	-	12.5	30.0	-	45.5	9.5	-	30
Moradabad											
Bhikanpur	1500	320	40.0	20.0	12.5	7.5	-	5.0	15.0	1.15	55
Dalpatpur	1100	300	30.0	7.5	5.0	15.0	-	40.0	2.5	1.15	52
Faridpur	1700	400	50.0	20.0	2.5	2.5	-	25.0	-	1.50	60
Firozpur	1800	400	40.0	-	7.5	15.0	15.0	15.0	7.5	1.45	50
Jahid Nagar	1800	460	65.0	-	10.0	-	-	20.0	5.0	1.70	75
Jatpura	2400	320	28.5	12.5	7.5	7.5	-	35.0	1.5	1.10	45
Ladpura	1500	300	25.0	22.5	17.5	-	-	25.0	10.0	1.20	50
Mainathai	1000	380	38.5	-	5.5	10.5	25.0	12.0	8.5	2.35	45
Maulagarh	2100	380	40.0	18.0	10.0	15.5	-	7.5	9.0	1.40	40
Munda Pandey	2000	360	45.0	12.5	5.5	25.0	-	7.5	4.5	1.25	52
Nasilpur	1700	400	40.0	5.0	7.5	7.0	30.0	5.5	5.0	1.60	44
Orchhi	1500	440	60.0	5.5	-	5.5	-	20.0	9.0	1.70	50
Sambhal	2200	460	40.0	-	12.5	-	40.0	7.5	-	2.80	60
Sahibabad	1500	400	50.0	25.0	10.0	-	-	10.0	5.0	1.50	45
Sirsi	2300	460	50.0	-	5.5	-	40.0	-	4.5	3.25	70
Badaun											
Bisauli	2200	360	25.0	12.5	7.0	-	7.5	30.0	18.0	1.50	55

Table 2 continued ... ...

Chaduiya	1000	-	-	-	5.5	-	50.0	7.5	37.0	-	40
Chaitrapur Madiya	1700	440	55.5	20.0	-	-	-	7.5	17.0	1.25	58
Dharampal Jirauli	1300	280	20.0	-	-	20.0	-	50.0	10.0	1.10	45
Dharampur	1000	200	48.5	11.5	-	-	40.0	-	-	0.85	35
Etauwa	2200	380	40.0	-	-	15.0	-	25.0	20.0	1.20	40
Faizganj	2000	420	50.0	20.0	5.0	5.0	20.0	-	-	1.50	65
Hasanpur	1400	460	45.0	2.5	40.0	-	-	12.5	-	1.75	50
Hatsa	800	180	30.0	5.5	5.5	-	40.0	-	19.0	0.85	40
Jaraga	2100	120	12.5	-	-	-	75.0	-	12.5	0.50	35
Mundia	800	100	28.5	7.5	5.0	8.5	35.0	-	14.5	0.75	45
Noorpur	1300	120	15.0	5.0	45.0	10.0	-	20.0	5.0	0.75	30
Sikrapur	700	280	40.0	2.5	5.5	-	50.0	-	2.0	1.15	40
Waheedpur	800	200	25.0	9.5	10.0	-	20.0	20.0	15.5	1.00	40
Lelai	2200	300	30.0	15.5	20.0	8.5	-	5.5	20.5	1.25	55
Bulandshahar											
Badarpur Nagla	1000	340	50.0	20.0	15.0	-	15.0	-	-	1.30	60
Chhatari	800	300	50.0	-	12.5	-	5.5	7.5	24.5	1.20	55
Jirauli	1500	240	42.5	5.0	10.0	10.0	2.5	15.0	15.5	1.00	45
Nibari Bangar	700	300	50.0	10.5	7.5	10.0	4.5	7.5	10.0	1.15	50
Simrauli	900	320	54.0	5.0	10.0	5.5	-	10.0	10.5	1.20	65
Udaypur Khurd	1000	320	45.0	5.0	10.0	10.0	-	10.0	20.0	1.25	40
Aligarh											
Jargawan	1000	360	45.0	5.0	-	10.0	-	15.0	25.0	1.30	60
Jirauli Dhoomsingh	1000	260	20.0	10.0	5.0	15.0	30.0	15.0	5.0	1.10	45
Malaypur	600	200	34.5	15.0	-	8.0	-	35.0	7.5	1.00	55
Raipur Dalpat pur	700	360	60.0	-	18.5	-	-	20.0	2.5	1.25	50
Etah											
Allah Nagar	700	40	5.5	25.0	15.0	15.0	-	30.0	9.5	0.20	30

[a]Others : - *Tylenchus* sp., *Xiphinema* sp. , *Longidorous* sp. , *Criconimoides* sp.
[b]Root-knot index: 0 = 0%, 1 = 1-25%, 2 = 26-50%, 3 = 51-75%, 4 = 76-100%.
[c]Percent roots and suckers infection by fungi.

that *Meloidogyne* spp. (99.0%) were present in all the localities surveyed, except locality Chhaduiya of Badaun district (Table 3 and 4). *Tylenchorhynchus* spp. was the next most widely distributed nematode (87.1%) followed by *Hop. indicus* (78.6%), *Hel. indicus* (76.9%), *P. thornei* (68.8%) and *R. reniformis* (39.8%) respectively. However, percent occurrence of these nematodes varied from district to district.

In Rampur, percent occurrence of *Meloidogyne* spp. (100%) was highest followed by *Tylenchorhynchus* spp. (93.3%), *P. thornei* (93.3%), *Hop. indicus* (86.7%), *Hel. indicus* (73.3%) and *R. reniformis* (26.7%), respectively.

In Bareilly, after *Meloidogyne* spp. (100%) second most dominant nematode was *Tylenchorhynchus* spp. (80.0%) followed by *Hel. indicus* (66.7%), *R. reniformis* (66.7%), *Hop. indicus* (53.3%) and *P. thornei* (40.0%), respectively.

In Moradabad, *Meloidogyne* spp. was found to occur in all the localities followed by *Hop. indicus/Tylenchorhynchus* spp. (93.3%), *Hel. indicus/P. thornei* (66.7%) and *R. reniformis* (33.3%), respectively.

In Badaun, percent occurrence of *Meloidogyne* spp. (93.3%) was highest followed by *Hel. indicus* (73.3%), *Hop. indicus* (66.7%), *Tylenchorhynchus* spp. (60.0%), *R. reniformis* (60.0%) and *P. thornei* (40.0%), respectively.

In Bulandshahar, *Meloidogyne* spp. and *Hop. indicus* were present in all the localities followed by *Tylenchorhynchus* spp./*Hel. indicus* (83.3%) and *P. thornei/R. reniformis* (66.7%), respectively.

In Aligarh, the percent occurrence of *Meloidogyne* spp. and *Tylenchorhynchus* spp. was 100% followed by *P. thornei* (75.0%), *Hel. indicus/Hop. indicus* (50.0%) and *R. reniformis* (25.0%), respectively.

In Etah district, *M. arvensis* was found to be cultivated on a large scale around Allah Nagar locality only, where J2 of *Meloidogyne* spp., *Tylenchorhynchus* spp., *P. thornei, Hel. indicus* and *Hop. indicus* were present in the soil.

Table 3: Average percent occurrence of plant-parasitic nematodes observed in March 2000 (Survey I).

Districts	*Meloidogyne* spp.	*Hel. indicus*	*Hop. indicus*	*P. thornei*	*R. reniformis*	*Tylenchorhynchus* spp.	Others[a]
Rampur (15)[b]	100.0	73.3	86.7	93.3	26.7	86.7	100.0
Bareilly (15)	100.0	60.0	53.3	40.0	60.0	80.0	86.7
Moradabad (15)	100.0	60.0	86.7	60.0	33.3	86.7	86.7
Badaun (15)	93.3	66.7	66.7	33.3	60.0	53.3	93.3
Bulandshahar(6)	100.0	66.7	100.0	66.7	50.0	83.3	100.0
Aligarh (4)	100.0	75.0	50.0	50.0	50.0	100.0	75.0
Etah (1)	100.0	100.0	100.0	100.0	0.0	100.0	-
Average	99.0	71.7	77.6	63.3	40.0	84.3	77.4

[a]Others: *Tylenchus* sp., *Xiphinema* sp., *Longidorous* sp., *Criconimoides* sp.

[b]Figures in parentheses are total number of samples.

Table 4: Average percent occurrence of plant-parasitic nematodes observed in May 2000 (Survey II).

Districts	*Meloidogyne* spp.	*Hel. indicus*	*Hop. indicus*	*P. thornei*	*R. reniformis*	*Tylenchorhynchus* spp.	Others[a]
Rampur (15)[b]	100.0	73.3	86.7	93.3	26.7	93.3	100.0
Bareilly (15)	100.0	66.7	53.3	40.0	66.7	80.0	73.3
Moradabad (15)	100.0	66.7	93.3	66.7	33.3	93.3	86.7
Badaun (15)	93.3	73.3	66.7	40.0	60.0	60.0	80.0
Bulandshahar(6)	100.0	83.3	100.0	66.7	66.7	83.3	83.3
Aligarh (4)	100.0	75.0	50.0	75.0	25.0	100.0	100.0
Etah (1)	100.0	100.0	100.0	100.0	0.0	100.0	100.0
Average	99.0	76.9	78.6	68.8	39.8	87.1	89.0

[a]Others: *Tylenchus* sp., *Xiphinema* sp., *Longidorous* sp., *Criconimoides* sp.

[b]Figures in parentheses are total number of samples.

1.2.2 Average population composition and nematode population in different districts

Data regarding average percent population composition of nematodes shows that during Survey I, the most dominant nematode genera was *Meloidogyne* spp. (20.7%) followed by *Tylenchorhynchus* spp. (19.9%), *Hel. indicus* (13.3%), *Hop. indicus* (11.1%), *R. reniformis* (10.3%) and *P. thornei* (9.9%), respectively (Table 5; Fig. 1A).

The population of all the nematodes was increased during Survey II. However, this ratio of increase in population was different among the species, as a result of which population composition was affected in comparison to Survey I. *Meloidogyne* spp. again dominated (32.7%) followed by *Tylenchorynchus* spp. (17.6%), *Hel. indicus* (11.1%), *R. reniformis* (9.4%), *Hop. indicus* (9.1%) and *P. thornei* (9.0%), respectively (Table 6; Fig. 1B).

Highest population of plant-parasitic nematodes (per 250 g soil) during the two surveys was 880 and 1947 at Bareilly followed by Moradabad (773 and 1740), Badaun (720 and 1433), Bulandshahar (417 and 983), Rampur (393 and 847), Aligarh (300 and 825) and Etah (200 and 700), respectively (Table 5 and 6).

1.2.3 Population of root-knot nematodes in roots/suckers

The population of root-knot nematodes was observed in the range of 0 - 180/g roots/suckers in March, which increased to 40 - 460/g roots/suckers during May (Table 1 and 2).

The population of *Meloidogyne* spp./g roots/suckers was highest in samples collected during Survey I and II from different localities of Moradabad (117 and 385) followed by Bulandshahar (73 and 303), Aligarh (60 and 295), Badaun (60 and 257), Bareilly (59 and 239), Rampur (49 and 156) and Etah (0.0 and 40), respectively.

1.2.4 Identification of root-knot nematode species from roots/suckers

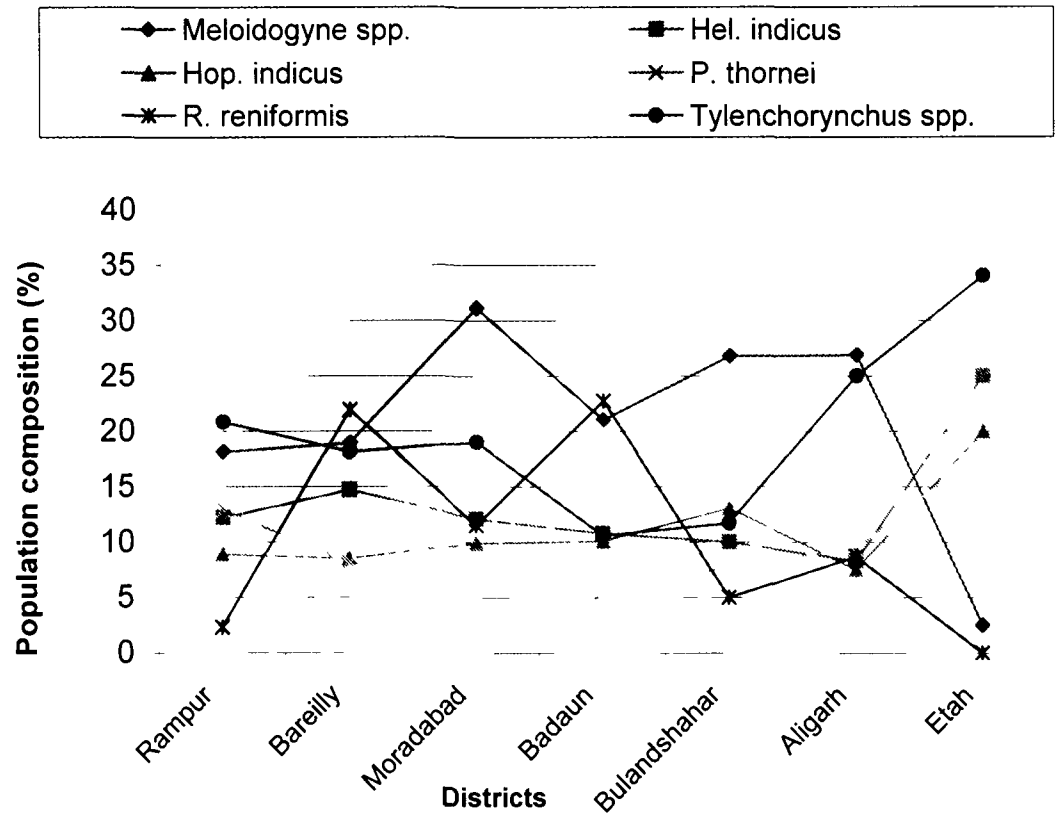
On the examination of perineal patterns of ten mature females excised from the roots/suckers of *M. arvensis,* collected from each and every locality

Table 5: Average population composition (%) of plant-parasitic nematodes and percent roots and suckers infection by fungi in the rhizosphere of *Mentha arvensis* observed in March 2000 (Survey I).

Districts	Nematodes population/ 250 g soil	*Meloidogyne* spp./g roots & suckers	*M. incognita*	*Hel. indicus*	*Hop. indicus*	*P. thornei*	*R. reniformis*	*Tylenchorhynchus* spp.	Others[a]	[b]Root-knot index	[c]Roots & suckers infection
Rampur (15)[d]	393	49	18.1	12.2	8.9	12.9	2.3	20.8	24.6	0.25	21.7
Bareilly (15)	880	59	18.9	14.7	8.5	8.1	21.9	18.1	9.9	0.28	22.0
Moradabad (15)	773	117	31.1	12.1	9.9	7.8	11.5	19.0	8.8	0.68	31.7
Badaun (15)	720	60	21.0	10.8	10.1	4.7	22.7	10.6	20.1	0.28	23.9
Bulandshahar (6)	417	73	26.8	10.0	13.1	7.9	5.0	11.7	18.6	0.35	30.0
Aligarh (4)	300	60	26.9	8.3	7.5	9.4	8.7	25.0	14.4	0.29	26.7
Etah (1)	200	-	2.5	25.0	20.0	18.5	-	34.0	-	-	15.0
Average	526.9	59.7	20.7	13.3	11.1	9.9	10.3	19.9	13.8	0.30	24.4

[a]Others: *Tylenchus* sp., *Xiphinema* sp., *Longidorous* sp., *Criconimoides* sp.
[b]Root-knot index: 0 = 0%, 1 = 1-25%, 2 = 26-50%, 3 = 51-75%, 4 = 76-100%.
[c]Percent roots and suckers infection by fungi.
[d]Figures in parentheses are total number of samples.

A.



B.

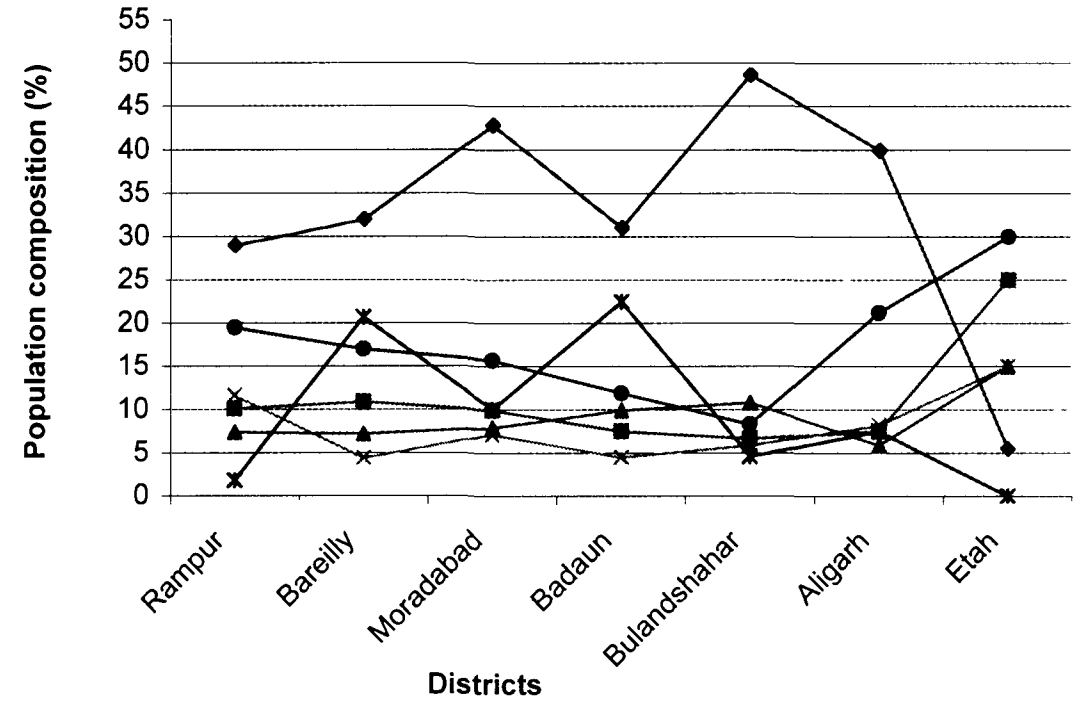


Fig.1: Average population composition of plant-parasitic nematodes in the rhizosphere of *M. arvensis* grown in various districts during March (A) and May (B) 2000 .

Table 6: Average population composition (%) of plant-parasitic nematodes and percent roots and suckers infection by fungi in the rhizosphere of *Mentha arvensis* observed in May 2000 (Survey II).

Districts	Nematode population/ 250 g soil	*Meloidogyne* spp./ g roots & suckers	*Meloidogyne* spp.	*Hel. indicus*	*Hop. indicus*	*P. thornei*	*R. reniformis*	*Tylenchorhynchus* spp.	Others[a]	[b]Root-knot index	[c]Roots & suckers infection
Rampur (15)[d]	847	156	29.0	10.1	7.4	11.6	1.8	19.5	20.0	0.73	39.0
Bareilly (15)	1947	239	32.0	10.9	7.2	4.4	20.7	17.0	6.0	0.98	41.3
Moradabad (15)	1740	385	42 8	9.9	7.9	7.1	10.0	15.7	5.8	1.67	52.8
Badaun (15)	1433	257	31.0	7.5	9.9	4.5	22.5	11.9	13.2	1.03	44.8
Bulandshahar (6)	983	303	48.6	6.7	10.8	5.9	4.6	8.3	14.9	1.18	52.5
Aligarh (4)	825	295	39.9	7.5	5.9	8.2	7.5	21.2	10.0	1.16	52.5
Etah (1)	700	40	5.5	25.0	15.0	15.0	-	30.0	9.5	0.20	30.0
Average	1198.8	208.6	32 7	11.1	9.1	9.0	9.4	17.6	11.3	0.96	44.7

[a]Others: *Tylenchus* sp., *Xiphinema* sp., *Longidorous* sp., *Criconimoides* sp.
[b]Root-knot index: 0 = 0%, 1 = 1-25%, 2 = 26-50%, 3 = 51-75%, 4 = 76-100%.
[c]Percent roots and suckers infection by fungi.
[d]Figures in parentheses are total number of samples.

during survey revealed that *M. incognita* (70%) was more prevalent than *M. javanica* (30%).

1.2.5 Root-knot index

Root-knot index was between 0.0 and 1.00 during Survey I, while during Survey II, it was between 0.0 and 3.25 (Table 1 and 2).

Root-knot index was highest at Moradabad (0.68 and 1.67) followed by Bulandshahar (0.35 and 1.18), Aligarh (0.29 and 1.16), Badaun (0.28 and 1.03), Bareilly (0.28 and 0.98), Rampur (0.25 and 0.73) and Etah (0.0 and 0.2) during Survey I and II, respectively (Table 5 and 6; Fig. 2A,B).

1.3 Isolation and identification of fungi from roots and suckers of *M. arvensis*

On the basis of cultural characters and microscopic studies, the various fungi isolated from the plant parts of *M. arvensis* were identified and further confirmed from ITCC, IARI, New Delhi as: *Fusarium pallidoroseum, F. solani, Rhizoctonia solani* and *S. sclerotiorum.*

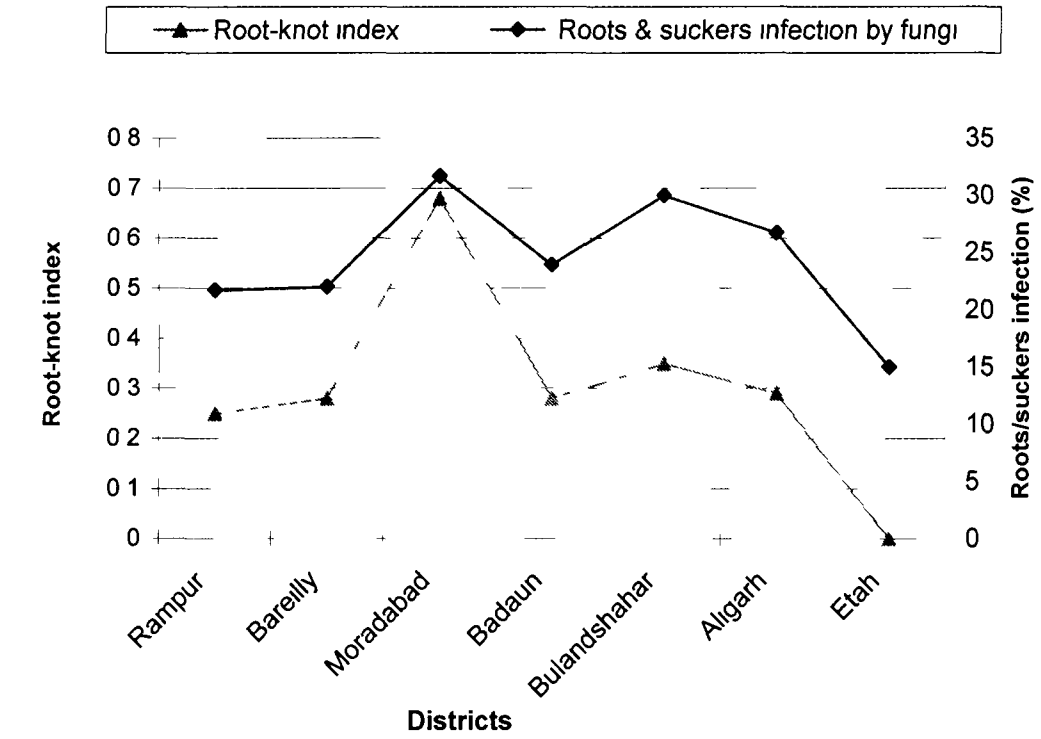
1.4 Percent roots/suckers infection by fungi

Data regarding percent roots/suckers infection of fungi ranged from 10 to 50% during Survey I and from 20 to 85% during Survey II (Table 1 and 2).

At all localities roots/suckers of *M. arvensis* were mainly infected with *S. sclerotiorum*, though in some cases other soil borne fungi were also found. Data showed that on an average the percent roots/suckers infection was 24.3% during Survey I, which was increased to 44.7% during Survey II (Table 5 and 6).

The highest percent roots/suckers infection was observed at Moradabad (31.7 and 52.8%) followed by Bulandshahar (30.0 and 52.5%), Aligarh (26.7 and 52.5%), Badaun (23.9 and 44.8%), Bareilly (22.0 and 41.3%), Rampur (21.7 and 39.0%) and Etah (15.0 and 30.0%) districts respectively, as shown in Table 5 and 6; Fig. 2A,B, respectively.

A.



B.

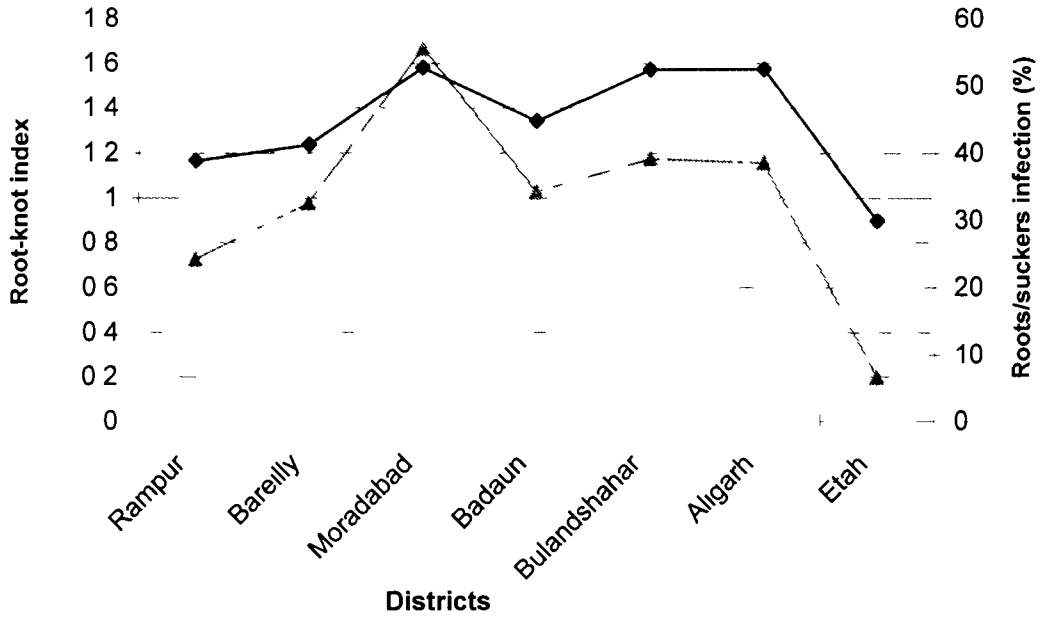


Fig. 2: Average root-knot index, infection by fungi in roots and suckers of *M. arvensis* in various districts during March (A) and May (B) 2000 .

DISCUSSION

Extensive surveys carried out in districts of Aligarh, Bulandshahar, Badaun, Bareilly, Etah, Moradabad and Rampur to assess the association of plant-parasitic nematodes and soil-borne fungi with *M. arvensis* revealed that during initial stages of plant growth visually there were no specific symptoms of nematode attack. Only few roots and suckers rarely showed root galling and egg masses. The appearance of water soaked areas due to *S. sclerotiorum* was frequently noticed on most of the suckers. Singh *et al.* (1997) also observed the similar symptoms on *M. arvensis* var. *piperescens*.

When the *M. arvensis* crop was at its maturity, patches of diseased plants showed symptomps of stunting with chlorotic and smaller leaves. Wilting of plants was also observed in many fields. The roots/suckers of such plants had severe galling with shiny egg masses. At several places, the roots/suckers were dark brown to black in colour and many were rotting. The decayed roots and suckers also contained fluffy growth of the mycelium in presence of good moisture in soil. During the survey at various locations, the black coloured sclerotia of *S. sclerotiorum* were also found attached to the infected suckers. Similar symptoms induced by *M. incognita*, *S. sclerotiorum*, *Fusarium pallidoroseum* and *F. solani*, have also been described in past (Sattar and Husain, 1978; Haseeb and Pandey, 1989; Singh *et al.* 1997).

In the present study, the *Meloidogyne* spp. were found to be the most widely distributed nematode followed by *Tylenchorhynchus* spp., *Hop. indicus*, *Hel. indicus*, *P. thornei* and *R. reniformis* respectively. The highest population of *Meloidogyne* spp. (soil and roots/suckers) and root-knot index was found in Moradabad followed by Bulandshahar, Aligarh, Badaun, Bareilly, Rampur and Etah districts respectively. The present findings confirm the reports of previous surveys on distribution and incidence of root-knot nematodes associated with mint cultivation (Haseeb and Pandey, 1989; Pandey *et al.*, 1992; Haseeb, 1992; 1994; Kumar and Singh, 1995; Shukla, 1997, 1998).

However, the population of plant-parasitic nematodes and severity of infection varied with district and locality probably because of the variation in susceptibility of different cultivars, soil types, and other cultural practices adopted by the farmers (Prot and Van Gundy, 1981; Swanson and Van Gundy, 1984; Windham and Barker, 1986).

The examination of perineal patterns of excised females from roots/suckers of *M. arvensis* collected during survey revealed the presence of *M. incognita* and *M. javanica*. However, *M. incognita* was observed to be more prevalent than *M. javanica*. These data verify and extended limited observations by Haseeb and Pandey (1989).

The highest percent root/sucker infection by fungi was observed in district of Moradabad followed by Bulandshahar, Aligarh, Badaun, Bareilly, Rampur and Etah, respectively. The identification of fungi isolated from the root/sucker of *M. arvensis* revealed the presence of *F. pallidoroseum, F. solani, R. solani* and *S. sclerotiorum*. The present observations hold similarity to the previous reports of Singh and Singh (1991), Singh *et al.* (1997) and Singh and Haseeb (1998), wherein the incidence of phytopathogenic fungi on mints was observed. A perusal of the literature revealed that no information is available on stolon decay caused by *S. sclerotiorum* on *M. arvensis* cv. Gomti (Bilgrami *et al.*, 1991; Singh and Haseeb, 1998). Thus, it appears to be a first record of *S. sclerotiorum* infecting *M. arvensis* cv. Gomti.

Survey studies conclusively indicated that *M. incognita* and *S. sclerotiorum* were consistently associated with *M. arvensis* in the mint growing areas of Western and Tarai region of U.P. Thus the information gathered during survey can be utilized in disease forecasting programmes of *Mentha* crop and ultimately this may be useful in developing the disease management packages.

Chapter 3

Pathogenicity Test

2. Effect of different initial inoculum levels of *M. incognita* on root-knot disease development, nematode multiplication, growth, oil yield and biochemical changes in plants of *M. arvensis* cv. Gomti

The ability to predict losses expected from a given nematode population is essential in making management decisions (Barker *et al.*, 1985). It is established that increasing nematode population densities can progressively affect crop performance, and there is a minimal threshold density below, which no measurable loss in yield occurs. Studies pertaining to such relationship have been conducted with several nematode-crop/plant systems. Most of the information are based on the studies carried out in pots or micro-plot experiments (Shaffie and Jenkins, 1963; Haseeb, 1983; Appel and Lewis, 1984; Di Vito *et al.*, 1985; Haseeb *et al.*, 1988, 1990, 1993, 1996, 1998; Haseeb and Butool, 1989; Haseeb and Pandey, 1990; Butool and Haseeb, 1992; Pandey *et al.*, 1992; Haseeb and Shukla, 1994, 1995, 1996, 2000b, 2001b, 2002).

REVIEW OF LITERATURE

Chitwood (1951) provided the first quantitative data, which showed that *M. hapla* suppressed the growth and yield of tomato. Shaffie and Jenkins (1963) demonstrated that 1,000 J2 of *M. incognita*/plant, stunted severely the growth of pepper, whereas, 1,000 J2 of *M. hapla*/plant caused only a little stunting in pot culture.

Singh and Mishra (1974) studied the effect of different initial inoculum levels viz. 0, 10, 100, 1,000 and 5,000 J2 of *M. javanica*/pot on the growth of sugarbeet. The results showed that the plants inoculated with higher levels of nematode inoculum showed unhealthy appearance. Development of the root system of sugarbeet was significantly reduced in plants receiving the highest J2 per pot. The intensity of gall formation increased with the increase in number of nematode. The root-knot index was 4.0 at the highest initial level of inoculum. Average weight of the roots (storage tissues) was observed to decline with an increase in the number of J2 inoculated. Sucrose content in the roots was low under all the treatments as compared to control.

Raut and Sethi (1980) determined the extent of damage to soybean variety Clark-63 by *M. incognita*. They observed progressive decrease in growth of soybean plant as the inoculum level of *M. incognita* increased. Significant reduction in top growth, root length and bacterial nodulation in comparison to uninoculated check plants was observed at an initial level of 1,000 J2 per kg soil or above. The 1,000 J2/pot was found to be the damaging threshold level.

Atu *et al.* (1983) determined the effect of different population levels of *M. incognita* ranging from 50 to 156, 250 eggs per plant on the growth of white guinea yam (*Dioscorea retundata*) cv. Iguwe. The economic threshold and economic injury level were fixed at 250 and 1,250 nematodes per plant, respectively at a soil temperature of 28 °C. The market value of yam was reduced by 40% and roots were galled heavily when the plants were inoculated with more than 1,250 nematodes/plant.

Appel and Lewis (1984) studied the effect of initial populations of *Hoplolaimus columbus* and *M. incognita* on the growth and yield of soybean cv. Davis in micro-plots in the year 1980 and 1981 and *H. columbus* in a field test in 1981. *M. incognita* suppressed the yield in micro-plots in both years and *H. columbus* in a field test in 1981. Maximum suppression of dry pod weight was 45 and 35% by *M. incognita* and *H. columbus* respectively. The reproduction rates for *M. incognita* and *H. columbus* was noticed inversely proportional to initial population level.

Di Vito *et al.* (1985) conducted two micro-plot experiments in 1981 and 1983 to study the effect of 0, 0.062, 0.125, 0.25, 0.50, 1, 2, 4, 8, 16, 32, 64, 128, 256 and 512 eggs and juveniles of *M. incognita* race-1/cm^3 soil on the yield of sweet pepper. They have suggested the tolerance limits of 2.2 and 0.165 eggs and juveniles/cm^3 soil and minimum yields of 5.8 and 20% of the controls were obtained in 1981 and 1983 respectively. The reproduction rate was maximum 274 and 1,498 at the lowest initial inoculum levels in 1981 and 1983 respectively. The population of the nematode declined rapidly after harvest, and only 13 and 6.5% of eggs and juveniles were detected in the soil after 1 and 6 months respectively.

Thakar *et al.* (1986) investigated the effect of 0, 10, 100, 1,000 and 10,000 J2 of *M. incognita*/kg soil on the growth of *Lathyrus sativus*. Maximum reduction in shoot length was observed at 10,000 J2, which was significantly different from all other treatments. Significant reduction in fresh shoot weight was observed at 1,000 and 10,000 J2 and it was at par with 100 J2. Root-knot indices were significantly increased with the increase in inoculum level.

Rai and Jain (1987) reported that the yield of *desi* (indigenous) cotton was significantly reduced in plants inoculated with 1,000 or more juveniles of *M. incognita*/plant, whereas, at and above 500 juveniles/plant was sufficient to decrease the yield in American cotton. However, qualitative characters were not affected significantly. Root-knot index was maximum (RKI = 5.0) at and above 1,000 juveniles/plant both in American and Desi cotton. There was a decrease in dry shoot weight with the increase in inoculum level.

Haseeb *et al.* (1988) studied the effects of different initial populations of *M. incognita* on the growth and oil yield of *O. basilicum* cv. French. Maximum reduction in dry weight of plant (54.57%) and oil yield (50.00%) was found at highest inoculum density (10,000 juveniles/plant). Maximum reproduction rate (54.16) of *M. incognita* was observed at Pi 50 juveniles/plant and was inversely proportional to initial population densities.

Azmi and Patil (1988) reported the decrease in length and fresh weight of shoot and root of the *Dolichos lablab* cv. Lignosus as the initial inoculum density of *M. incognita* increased. Significant reduction in growth occurred at initial inoculum of 1,000 nematodes/500 cc soil. Final nematode population was highest at the initial inoculum level of 1,000 nematodes.

Midha and Trivedi (1988) determined the influence of 0, 10, 100, 1,000 and 10,000 J2 of *M. incognita* on the growth of coriander. Plant growth was increased in plants inoculated with 10 J2/plant. Highest reduction in all plant growth characters was obtained at 10,000 J2/plant. The reproduction factor was negatively correlated with the inoculum density.

Haseeb and Butool (1989) established the pathogenicity of *M. incognita* on *O. sanctum*. They reported that the fresh and dry weight and the total oil yield of *O. sanctum* were negatively correlated with the initial inoculum levels, whereas, the nematode reproduction was density dependent.

Haseeb and Pandey (1990) established the pathogenicity of *M. incognita* on *Artemisia pallens* and reported that every increase in initial inoculum level of the nematode resulted in corresponding decrease in shoot height, root length, fresh/dry root-shoot weight and oil yield. Highest decrease in fresh (63.87%) and dry weight (70.57%) of the plant was observed at highest inoculum level (15,000 juveniles/kg soil). The root-knot development was directly proportional to the increase in nematode population.

Haseeb *et al.* (1990) determined the influence of 0, 50, 250, 500, 2,500, 5,000, 7,500, 10,000, 12,500 and 15,000 J2 of *M. incognita*/kg soil on the root-knot disease development, nematode multiplication, growth of *H. niger* plant, total alkaloid yield, physiological responses (total chlorophyll content, CO_2 exchange rate) and concentration of sodium, potassium, iron, manganese, copper and zinc 90 days after inoculation. They have reported that with the increase in inoculum levels, there was a corresponding decrease in fresh and dry plant weight, total alkaloid yield, total chlorophyll content, photosynthetic rate, sodium, potassium, iron, manganese, copper and zinc concentrations in roots and shoots except for sodium and potassium, which decreased in shoot. The greatest percent reduction in all the characters was obtained when plants were inoculated with the highest nematode inoculum. The relation between initial inoculum density and final nematode population showed a maximum reproduction rate of *M. incognita* at Pi of 50 J2/plant.

Butool and Haseeb (1992) determined the effect of *M. incognita* on the yield of *Trachyspermum ammi*. The increase in inoculum potential of the nematode resulted in corresponding decrease in plant length, fresh and dry weight of root and shoot. The highest root-knot galling on *T. ammi* was found at 2500 juveniles/kg soil, but with further increase in nematode inoculum the root-knot galling did not increase further.

Pandey *et al.* (1992) carried out an experiment in green house to determine the effect of graded population densities of *M. incognita* on the growth, yield of *M. arvensis* var. MAS-1 and reproduction of the nematode. Studies indicated that an increase in initial population densities of nematode causes a considerable reduction in root-shoot length, fresh and dry weight, chlorophyll a, b and total, rate of photosynthesis and oil yield of the crop. The rate of nematode reproduction was found to be density dependent.

Haseeb *et al.* (1993, 1996) established the pathogenicity of *M. incognita* on *O. basilicum* and *O. canum*. They found that the growth and oil yield of both species of *Ocimum* was reduced significantly at minimum initial inoculum densities of *M. incognita* (500 J2). Reduction in growth and oil yield was reported directly proportional to Pi while reproduction rate of the nematode was inversely proportional to Pi levels. However, root-knot development was found directly proportional to Pi levels.

Kumar and Singh (1997) studied the influence of different initial population densities of *M. incognita* on the growth of *M. arvensis* cv. Shivalik. They found that there was progressive decrease in length and weight of shoot and root/sucker with the increase in the initial nematode inoculum.

Goyal and Trivedi (1999) established the pathogenicity of *M. incognita* on *Antirrhinum majus* and *Dianthus barbatus*. They reported that the shoot length, fresh and dry weight of shoot and root in both the plants significantly reduced at or above Pi of 10 J2. An increase in the initial inoculum level from 10 to 10,000 J2 resulted in a progressive increase in number of galls as well as nematode multiplication.

Haseeb and Shukla (2000a, 2001b) studied the effect of *P. thornei* and *M. incognita*, alone and in combinations on the growth and oil yield of *M. arvensis* and *M. cardiaca*. They observed the influence of all the Pi of *P. thornei* and *M. incognita* on the growth and oil yield of *M. arvensis* was inversely proportional and highly significant. *M. incognita* suppressed plant growth more severely than *P. thornei* at equal Pi. Final nematode population of *P. thornei* and *M. incognita*

was increased with the increase in Pi, whereas, rate of reproduction decreased with the increase in Pi.

Khan *et al.* (2001) studied the effect of initial inoculum levels of *M. incognita* race-2 on onion. They reported significant reduction in foliage and weight of onion bulbs of all the inoculated plants as compared to uninoculated plants. The highest nematode multiplication rate was observed at the 10 J2/500 cm^3 soil.

Biochemical changes in plants

Plant-parasitic nematodes are known to affect the plant metabolic processes such as water absorption, translocation of nutrients, growth hormones, photosynthesis, respiration, transpiration and biochemical alterations (Rhode, 1965; Brueske and Dropkin, 1973; Loveys and Bird, 1973; Wallace, 1974; Franco, 1980; Melakeberhan *et al.*, 1984, 1985; Haseeb *et al.,* 1990; Haseeb and Shukla, 1994, 1995, 1996).

Owens and Specht (1966) reported that little more than 50% reduction of free sugars was found in galled tomato roots infected with *M. incognita* and *M. incognita acrita* over healthy ones.

Feldman and Hanks (1968) reported that there was 27.30% increase in the bound phenolics in *R. similis* infected roots of tolerant cultivars of citrus and 16.34% decrease in susceptible ones. Keoboorueng (1971) reported that infection of rice seedlings with *Hirschmanniella oryzae* caused an increase in phenolic compounds in the roots.

Epstein (1972) studied the effect of *Longidorus africanus* on phenol content in roots of *Bidens tripartita* and *Vitis vinifera*. He concluded that the phenols of the infected root tips differ from those of the healthy tips both quantitatively and qualitatively. The amount of phenols in infected roots was more than double as compared to healthy roots.

Wang and Bergeson (1974) noted that the glucose was absent in root exudates of galled tomato roots infected with *M. incognita*, whereas, fructose, sucrose and polysaccharide were increased by 133-836 percent over control.

Rezk *et al.* (1976) found that phenolic contents of either healthy or *M. javanica* infected corn and rice roots consistently increased with plant age and such increase was much greater in resistant plants than in susceptible ones. Friedman and Rhode (1977) reported that the higher concentration of free phenol was found in the leaves of tomato cvs. Manalucie, Rutgers and Valiant infected with *P. penetrans* than in control.

Van Gundy *et al.* (1977) reported that during the first 14 days following infection of tomato plants by *M. incognita*, carbohydrates were the major constituents in root exudates. Singh *et al.* (1978) reported that in plants of *Solanum melongena* cv. Punjab chamkila inoculated with the *M. incognita* (100 J2/pot) resulted in an increase in protein, amino acids, proline and phenol content in roots. Whereas, starch content was decreased in infected roots and the magnitude was more pronounced up to 60 days after inoculation resulting in 32-26% decrease in starch content.

Nasar *et al.* (1980) studied the effect of *M. incognita* and *M. javanica* on the mineral, amino acid and carbohydrate concentrations of bitter almond and Nemaguard peach rootstocks. The concentration of total carbohydrates in Nemaguard peach was unaffected by nematode infestation. *M. incognita* significantly decreased the total carbohydrates concentration of both roots and leaves of bitter almond and *M. javanica* significantly decreased the total concentration of carbohydrates in roots only.

Goel *et al.* (1982) showed that infection with *M. javanica* enhanced the levels of O-dihydric phenols, total phenols and I.A.A. in infected plants of *Carica papaya*. Basu and Sukul (1983) and Sarna and Trivedi (1987) reported a decrease in sugar in the roots of *Hibiscus esculentus* and *Cicer arietinum* respectively infected with *M. incognita* as compared to uninoculated controls.

Melakeberhan *et al.* (1985) determined the morphological and physiological characteristics in *Phaseolus vulgaris* in destructive and non-destructive assays in 3, 8, 15, 21 and 28 days after inoculation with 0, 2000, 4000 and 8000 juveniles of *M. incognita*. They concluded that the chlorophyll content and photosynthetic rate for all treatments decreased with time. Further,

significant reduction in overall nutrient elements per plant was obtained in nematode inoculated plants.

Melakeberhan *et al.* (1986) studied the influence of single generation of *M. incognita* on the physiological process and morphology of French bean plants inoculated with 0, 2,000, 4,000 and 8000 juveniles of *M. incognita* at different stages of growth. Chlorophyll content, plant dry weight and the number of buds, flowers, pods and seeds were significantly lower in infected plants than in the control.

Upadhyay and Banerjee (1986) studied the quantitative changes in sugar contents in root and stem of chickpea var. K-850 infected with *M. javanica.* Sugar content showed a decreasing trend as compared to uninoculated control. However, the increase was dependent upon the degree of infection.

Ferraz *et al.* (1989) reported the effect of *M. incognita* (600 J2/pot) on chlorophyll content and leaf senescence in *Piper nigrum.* They observed that the chlorophyll content of the leaves was significantly reduced in nematode inoculated plants than in uninoculated control.

Tiyagi and Alam (1990) studied the effect of 100, 1,000 or 10,000 *M. incognita, R. reniformis* or *T. brassicae*/plant on water absorption, chlorophyll content and growth of chickpea. There was a significant reduction in plant growth, water absorption and chlorophyll content irrespective of inoculum level and even the nematode species. *M. incognita* has the highest effect on all the test parameters of plants.

Patel and Patel (1990) studied the effect of *T. vulgaris* on the peroxidase and polyphenol oxidase activity and total phenol content in roots of bidi tobacco cv. Anand-119 and Gujarat tobacco-5. They noted an increase in peroxidase activity and total phenol content, however, there was a decrease in polyphenol oxidase activity with the increase in inoculum levels of the nematode.

Singh (1991) reported that amino acids, proteins and reducing sugars were increased in roots of *Capsicum frutescens* inoculated with *M. incognita* (1,000 J2/pot). Whereas, non-reducing sugars, total sugars and starch content

was decreased. Sharma and Trivedi (1992) reported decrease in total sugar, non-reducing sugars and starch in the roots of *Trigonella foenun-graecum* susceptible and resistant cultivars inoculated with *M. incognita* as compared to healthy roots of both the varieties. Whereas, free amino acids, proline, phenolic contents were increased in infected roots of both the varieties.

Haseeb *et al.* (1993) determined the effect of 0, 50, 250, 500, 2,500, 5,000 and 10,000 freshly hatched J2 of *M. incognita* on the growth, physiology, nutrient concentration and alkaloid yield of *Hyoscyamus albus*. With an increase in initial inoculum densities of nematodes resulted in a corresponding decrease in total chlorophyll content, photosynthetic rate and total alkaloid yield, sixty days after inoculation. The highest decrease in total chlorophyll content, photosynthetic rate and alkaloid yield was 40.0, 68.9 and 86.9 percent respectively in plants inoculated with 10,000 J2/kg soil. Whereas, the lowest reduction in all three parameters was 7.5, 2.5 and 10.2 percent respectively in plants inoculated with 50 J2/kg soil.

Mahmood and Siddiqui (1993) reported that the phenolics and amino acids contents were significantly high in *R. reniformis* inoculated in tolerant cultivars of tomato and were lowest in highly susceptible cultivars. They found a positive correlation between degree of nematode resistance and amounts of phenolics and amino acids in tomato cultivars.

Sharma *et al.* (1996) reported that the pea roots infected with *M. incognita* showed an increase in total and reducing sugar content, whereas, starch and non-reducing sugar were decreased. Mohanty *et al.* (1997) observed higher concentration of total sugar and amino acids in roots of greengram inoculated with *M. incognita* and/or rhizobium. However, chlorophyll content in leaves was reduced only in plants inoculated with nematode.

Poornima and Vadivelu (1999) reported lower levels of protein, carbohydrates, chlorophyll a, b and total and rhizome curcumin in plants of *Curcuma longa* cv. BSR-1 and PTS-10 inoculated with 1,000 J2 of *M. incognita* as compared to healthy ones.

Mohanty *et al.* (2001) determined the effect of *M. incognita* on the biochemical changes in roots of French bean. They noted the higher concentration of amino acids, amides, phenolic compounds and total sugar content in the nematode and/or *Rhizobium* inoculated roots as compared to uninoculated roots of healthy plants.

MATERIALS AND METHODS

2.1 Maintenance of planting material of *M. arvensis* cv. Gomti

Planting material of *M. arvensis* cv. Gomti was obtained from Central Institute of Medicinal and Aromatic Plants, Lucknow, India. Ten cm long disease free aerial portions of *M. arvensis* cv. Gomti were cut and planted singly into 30-cm-diameter clay pots containing steam sterilized soil and farmyard manure (5:1) mixture. Pots were kept on a concrete platform and irrigated as needed. Plants were observed regularly for insect pests and diseases.

2.2 Maintenance and production of culture of *M. incognita.*

During the identification of the *Meloidogyne* species infesting roots/suckers of *M. arevensis* (described in 1.5.3), egg masses were picked before excising and identifying the species. After identification, egg masses of *M. incognita* were surface sterilized in 1:500 aqueous solution of sodium hypochlorite for 5 minutes and a single egg mass was transferred to a small coarse sieve lined with double layer of tissue paper, earlier placed in a petri plate containing water. The petri plates were incubated at room temperature (27 ± 5 °C) (den Ouden, 1958). Seedlings of eggplant, grown in autoclaved soil were inoculated with second-stage juveniles (J2). Later, eggplant seedlings were inoculated with progeny of the single egg mass from time to time to supply inoculum for experiments.

2.3 Transplanting and inoculation

Five cm length of healthy suckers of *M. arvensis* cv. Gomti obtained from pots which were maintained for the purpose of planting materials, were transplanted singly into 30-cm-diameter clay pots containing 5 kg steam

sterilized soil and farm yard manure (5:1) mixture. Pots were kept on concrete platform for the establishment of plants. Plants were irrigated as needed. At the 4th leaf stage, four to five 5-cm-deep, holes were made around the test plants within a radius of 1.5 cm and pre-determined number of *M. incognita* J2 in aqueous suspension (0, 500, 1,000, 2,500, 5,000, 10,000, 15,000, 20,000, and 25,000) were poured into the holes separately in each pot, using a sterilized pipette. The holes were then plugged gently with soil. There were five replicates for each treatment. The experiment was laidout as a completely randomized block design

2.4 Recording of data

2.4.1 Plant growth

One hundred days after inoculation, plants were carefully uprooted from pots and roots/suckers were washed in running tap water to remove the adhering soil particles. Excess water was removed with blotting paper. Plant growth was determined by measuring shoot height, fresh and dry weights of shoot and roots/suckers. For determining dry weight, the shoot and roots/suckers were dried in an oven at 60 °C for 24 hours. The percent reduction in plant growth over uninoculated control was also calculated.

2.4.2 Estimation of nematode population

At experiment termination, soil and root/sucker samples were taken from each and every replicate for the estimation of nematode population. Isolation of nematodes from soil and roots/suckers was done as described earlier in 1.5.1 and 1.5.2, respectively. Root-knot index was graded on 0-4 scale as mentioned in 1.3.

2.4.3 Estimation of chlorophyll

Chlorophyll (a, b and total) content of the third leaf from the apex was estimated according to the method of Arnon (1949). Fresh leaf (0.2 g) samples from each replicate were ground separately in a mortar and pestle. Leaf tissue was homogenized in 80% acetone and the homogenate was filtered through Whatman filter paper no. 1 into a volumetric flask. During grinding calcium carbonate was added to the sample to avoid loss of chlorophyll. The process was

repeated thrice and the final volume (v) of extract was brought to 25 ml. The absorbance (A) of extract was recorded at 645 and 663 nm on spectrophotometer and the total chlorophyll, chlorophyll a and chlorophyll b were calculated by the following formula:

Total chlorophyll = (20.2 x A645) + (8.02 x A663) x factor

Chlorophyll a = (12.3 x A663) - (0.86 x A645) x factor

Chlorophyll b = (19.3 x A645) - (3.60 x A663) x factor

$$\text{Factor} = \frac{\text{Volume (v)}}{1000 \times \text{leaf weight (g)}}$$

2.4.4 Estimation of sugar

Total sugar content of the third leaf from the apex was estimated according to the method of Yemm and Willis (1954). Fresh leaf (0.5 g) samples from each replicate were transferred separately to 10 ml boiling ethanol (80%). The extract was clarified by adding 10 ml of 10% neutral lead acetate with stirring and filtered after 15 minutes. To this filtrate 5 ml of 10% potassium oxalate was added in order to precipitate lead. The solution was filtered and final volume was made up to 50 ml. One ml of this filtrate was placed into a test tube and 5 ml anthrone reagent added slowly while keeping the tube in ice. The tubes were placed in boiling water bath for 15 minutes and then rapidly cooled and the O.D. was read at 620 nm on a spectrophotometer. Sugar content was calculated from a glucose standard curve.

2.4.5 Estimation of total phenol

Total phenol content of the third leaf from the apex was estimated according to the method of Swain and Hill (1959). Fresh leaf (0.5 g) samples from each replicate were separately extracted with 30 ml methanol and the process repeated thrice each time decanting supernatant. The extract was pooled and evaporated to dryness. The residue was dissolved in 0.5 ml methanol and volume was made to 25 ml with distilled water. One ml of extract was diluted to 6 ml with distilled water and 0.5 ml Falincio Calteu reagent (1:1 diluted) was

added (Keith *et al.*, 1958). After 3 minutes, 1 ml of 35% Na_2CO_3 was added to the reaction mixture and final volume was made up to 10 ml. The tubes were kept in darkness for 30 minutes and afterwards O.D. was recorded at 600 nm on a spectrophotometer. The phenol content was calculated from a standard curve of gallic acid.

2.4.6 Extraction of oil

The essential oil content was determined by hydro-distillation of fresh herb using Clevenger apparatus (Clevenger, 1928). From each replicate hundred gram chopped fresh herb was filled into the round bottom flask of the apparatus and 200 ml water was added. The flask was kept on a heating mantel at 85.5 °C. As water started to boil, oil from herb evaporated, passed through a condenser, and drops of oil were accumulated on the water filled in measuring tube of the apparatus. The process was run for two hours and the volume of oil was measured in ml and percent oil yield was calculated on fresh herb basis.

2.5 Statistical analysis of data

The data were analyzed by least significant difference (L.S.D.) test at probability of 0.05 and 0.01 to identify significant effect of a treatment (Dospekhov, 1984).

RESULTS

Different initial inoculum levels of *M. incognita* on *M. arvensis* cv. Gomti reveals that, in general, reduction in growth parameters increased with the corresponding increase in initial inoculum levels (Table 7; Fig. 3; Plate 5). The significant ($P \leq 0.05$) reduction in shoot height, plant fresh and dry weights were observed at the lowest inoculum level (500 J2/5 kg soil) as compared to uninoculated control. The maximum reduction in shoot height, shoot-root fresh and dry weights was 43.4, 45.0, 48.9, 45.7 and 49.6%, respectively, at the highest initial inoculum level (25,000 J2/5 kg soil) as compared to uninoculated control. Analyses of data indicated that differences in all plant growth parameters among different inoculum levels were significant ($P \leq 0.05$).

Table 7: Effect of different initial inoculum levels of *Meloidogyne incognita* on root-knot disease development, nematode multiplication and growth of *Mentha arvensis* cv. Gomti[a].

Initial inoculum levels	Shoot height (cm)	Plant fresh weight (g)			Plant dry weight (g)			Final nematode population			Repro-duction factor	[b]Root-knot index
		Shoot	Roots & suckers	Total	Shoot	Roots & suckers	Total	Roots & suckers	Soil (5 kg)	Total		
0	81.8	147.4	134.0	281.4	35.0	25.8	60.8	-	-	-	-	-
500	78.7 (3.8)[c]	140.0 (5.0)	125.5 (6.3)	265.5 (5.6)	33.0 (5.7)	24.0 (7.0)	57.0 (6.2)	22590	18000	40590	81.18	0.87
1000	76.2 (6.8)	134.8 (8.3)	120.5 (10.1)	255.3 (9.3)	31.8 (9.1)	23.0 (10.8)	54.8 (9.9)	31330	30000	61330	61.33	1.00
2500	72.5 (11.4)	120.5 (18.2)	106.0 (20.9)	226.5 (19.5)	28.5 (18.6)	20.3 (21.3)	48.8 (19.7)	36040	42000	78040	31.22	1.25
5000	67.7 (17.2)	110.8 (24.8)	97.7 (27.1)	208.5 (25.9)	26.1 (25.4)	18.6 (27.9)	44.7 (26.5)	44942	52000	96942	19.39	1.87
10000	60.5 (26.0)	103.2 (30.0)	89.2 (33.4)	192.4 (31.6)	24.2 (30.8)	17.0 (34.1)	41.2 (32.2)	48168	60000	108168	10.82	2.25
15000	55.0 (32.8)	93.0 (36.9)	83.0 (38.0)	176.0 (37.4)	22.0 (37.1)	15.9 (38.4)	37.9 (37.7)	51460	68000	119460	7.96	2.65
20000	49.0 (40.1)	87.7 (40.5)	74.8 (44.2)	162.5 (42.2)	20.7 (40.8)	14.5 (43.8)	35.2 (42.1)	52302	74000	126302	6.32	2.85
25000	46.3 (43.4)	81.0 (45.0)	68.5 (48.9)	149.5 (46.9)	19.0 (45.7)	13.0 (49.6)	32.0 (47.4)	53430	80000	133430	5.34	3.00
L.S.D. $_{0.05}$	2.5	5.0	4.3	10.2	1.1	0.9	1.0	76.9	2161.2	10219.1	1.81	0.12
L.S.D. $_{0.01}$	3.4	7.8	5.8	13.7	1.5	1.2	1.3	103.7	2917.0	15192.3	2.38	0.17

[a]Each value is an average of five replicates.
[b]Root-knot index: 0 = 0%, 1 = 1-25%, 2 = 26-50%, 3 = 51-75%, 4 = 76-100%.
[c]Figures in parentheses are percent reduction over uninoculated control.

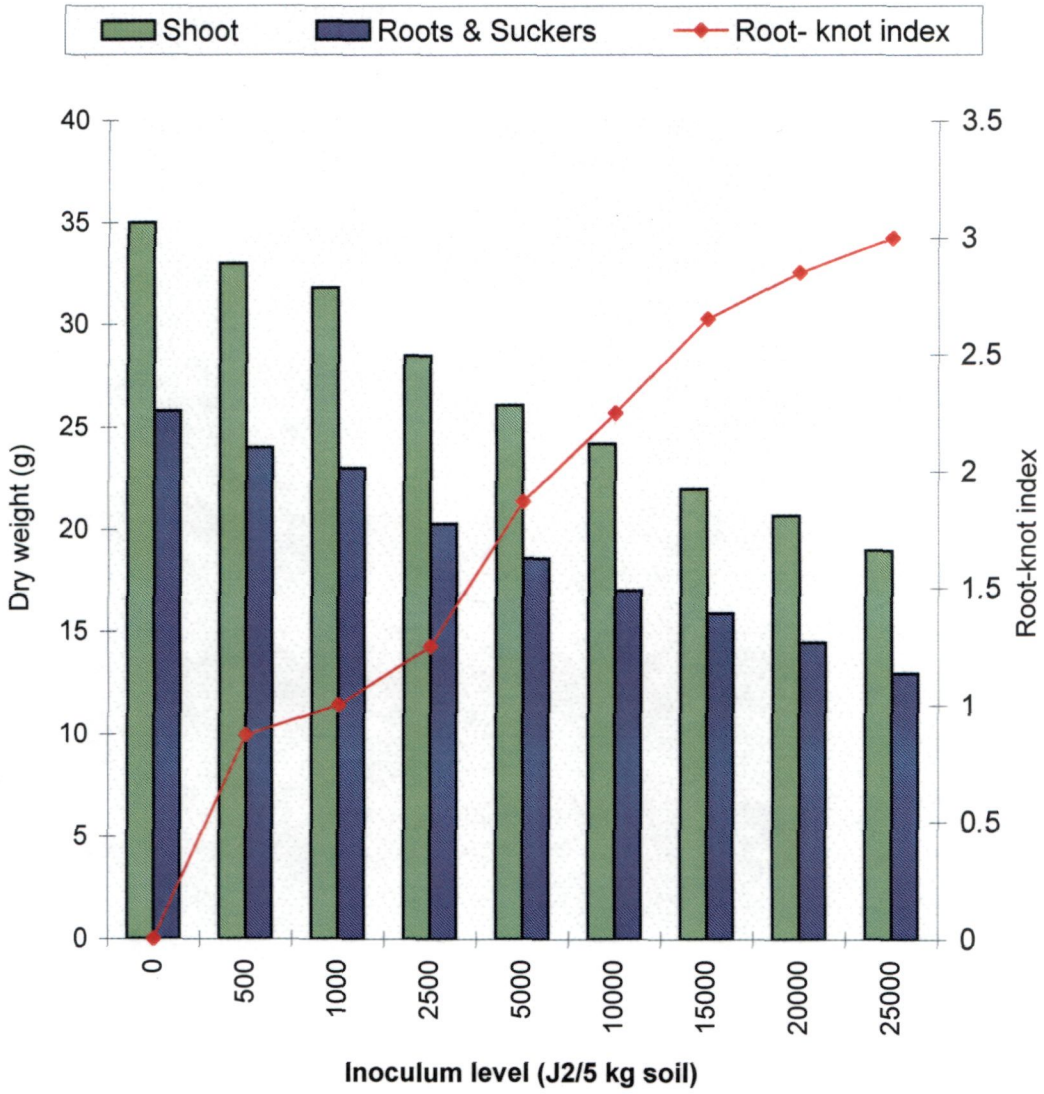


Fig. 3: Effect of different inoculum levels of *M. incognita* on root-knot disease development and growth of *M. arvensis* cv. Gomti.

Root-knot index:- 0 = 0%, 1 = 1-25%, 2 = 26-50%, 3 = 51-75%, 4 = 76-100%

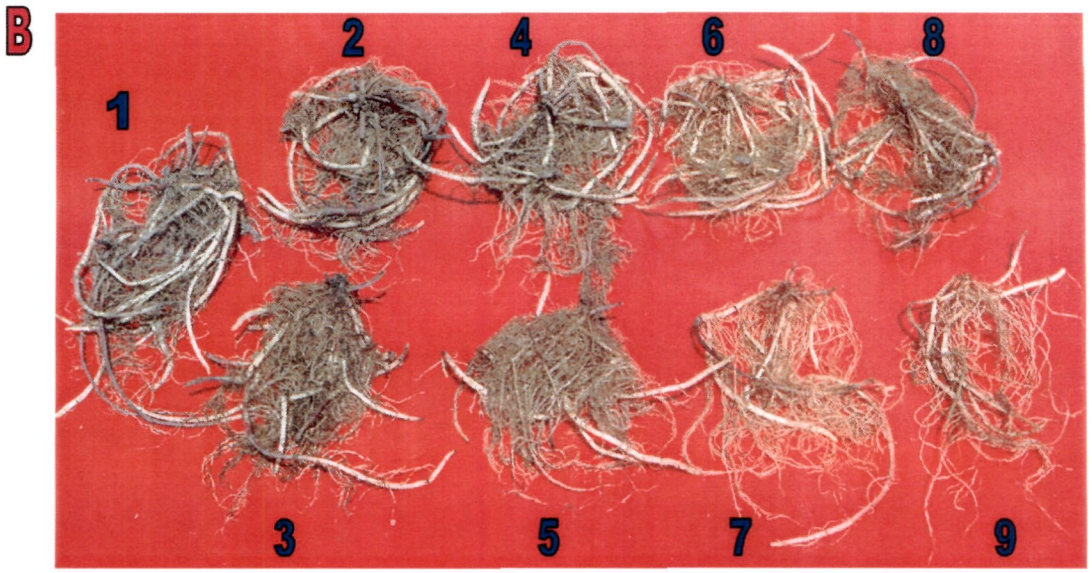


Plate 5: Effect of different inoculum levels of *M. incognita* on the aerial growth (A) and on roots and suckers development (B) of *Mentha arvensis* cv. Gomti.

1 = Uninoculated control	4 = 2500 J2/pot	7 = 15000 J2/pot
2 = 500 J2/pot	5 = 5000 J2/pot	8 = 20000 J2/pot
3 = 1000 J2/pot	6 = 10000 J2/pot	9 = 25000 J2/pot

The final population of nematode (Pf) in roots/suckers and soil and root-knot index increased with the corresponding increase in initial nematode population, while, the reproduction factor (Rf) decreased. Maximum Pf (1,33,430) and root-knot index (3.00) were observed at the highest Pi, whereas, maximum Rf (81.18) was observed at minimum Pi. Analyses of data indicated that differences in Pf, Rf and RKI among different Pi were significant ($P \leq 0.05$).

An increase in Pi corresponded to a decrease in oil yield, chlorophyll (a, b and total), total phenol and total sugar content of the plant (Table 8; Fig. 4). Maximum reduction in oil yield, chlorophyll a, chlorophyll b, total chlorophyll, total phenol and total sugar content of fresh leaves was 42.5, 46.6, 42.4, 45.4, 47.0 and 45.9%, respectively, observed at the highest inoculum level as compared to uninoculated control. Analyses of data indicated that there was no difference ($P \leq 0.05$) in oil yield between Pi of 500 and 1,000 J2/5 kg soil. At lowest Pi the reduction in chlorophyll a content was non-significant ($P \leq 0.05$) while chlorophyll b was reduced significantly ($P \leq 0.05$). With both chlorophyll a and chlorophyll b no differences ($P \leq 0.05$) were observed between the Pi of 10,000 and 15,000 J2/5 kg soil and between 20,000 and 25,000 J2/5 kg soil, whereas, the effect of all inoculum levels on total chlorophyll, total phenol and total sugar was always significant ($P \leq 0.01$).

DISCUSSION

The pathogenicity tests of *M. incognita* on *M. arvensis* cv. Gomti clearly indicated the evidence of its potentiality in reducing the plant height, fresh/dry weight, oil yield, chlorophyll, total sugar, and total phenol content in infected plants. In general there was a positive relationship between the initial inoculum levels (Pi) of *M. incognita* and reduction in all the test parameters and a negative relationship between initial inoculum densities and rate of nematode multiplication (Rf). Present findings are the first to record effect of *M. incognita* on *M. arvensis* cv. Gomti and quite similar to previous studies carried out on other varieties of *M. arvensis* and other crops (Haseeb *et al.*, 1988, 1990, 1993, 1996, 1998; Pandey *et al.*, 1992; Kumar and Singh, 1997).

Table 8: Effect of different initial inoculum levels of *Meloidogyne incognita* on oil yield and biochemical changes in plants of *Mentha arvensis* cv. Gomti[a].

Initial inoculum levels	Oil yield (ml/100 g fresh herb)	Chlorophyll content (mg/g fresh leaves)			Total phenol (mg/g fresh leaves)	Total sugar (mg/g fresh leaves)
		Chl a	Chl b	Total chl		
0	0.80	1.31	0.66	1.98	13.50	15.90
500	0.76 (5.0)[b]	1.25 (4.58)	0.63 (4.55)	1.89 (4.54)	12.75 (5.55)	15.12 (4.90)
1000	0.74 (7.5)	1.16 (11.45)	0.58 (12.12)	1.75 (11.62)	12.20 (9.63)	14.50 (8.80)
2500	0.71 (11.25)	1.09 (16.79)	0.53 (19.70)	1.63 (17.68)	11.05 (14.81)	13.85 (12.89)
5000	0.64 (20.00)	1.00 (23.66)	0.49 (25.76)	1.50 (24.24)	10.00 (25.92)	12.35 (22.33)
10000	0.58 (27.5)	0.89 (32.06)	0.44 (33.33)	1.34 (32.32)	9.40 (30.34)	11.25 (29.24)
15000	0.53 (33.75)	0.84 (35.88)	0.43 (34.85)	1.28 (35.86)	8.75 (35.18)	10.45 (34.28)
20000	0.50 (37.50)	0.77 (41.22)	0.39 (40.91)	1.17 (40.91)	8.20 (39.26)	9.75 (38.68)
25000	0.46 (42.50)	0.71 (46.56)	0.38 42.42)	1.08 (45.45)	7.15 (47.04)	8.60 (45.90)
L.S.D. $_{0.05}$	0.03	0.07	0.03	0.05	0.22	0.14
L.S.D. $_{0.01}$	0.04	0.09	0.05	0.07	0.29	0.19

[a]Each value is an average of five replicates
[b]Figures in parentheses are percent reduction over uninoculated control

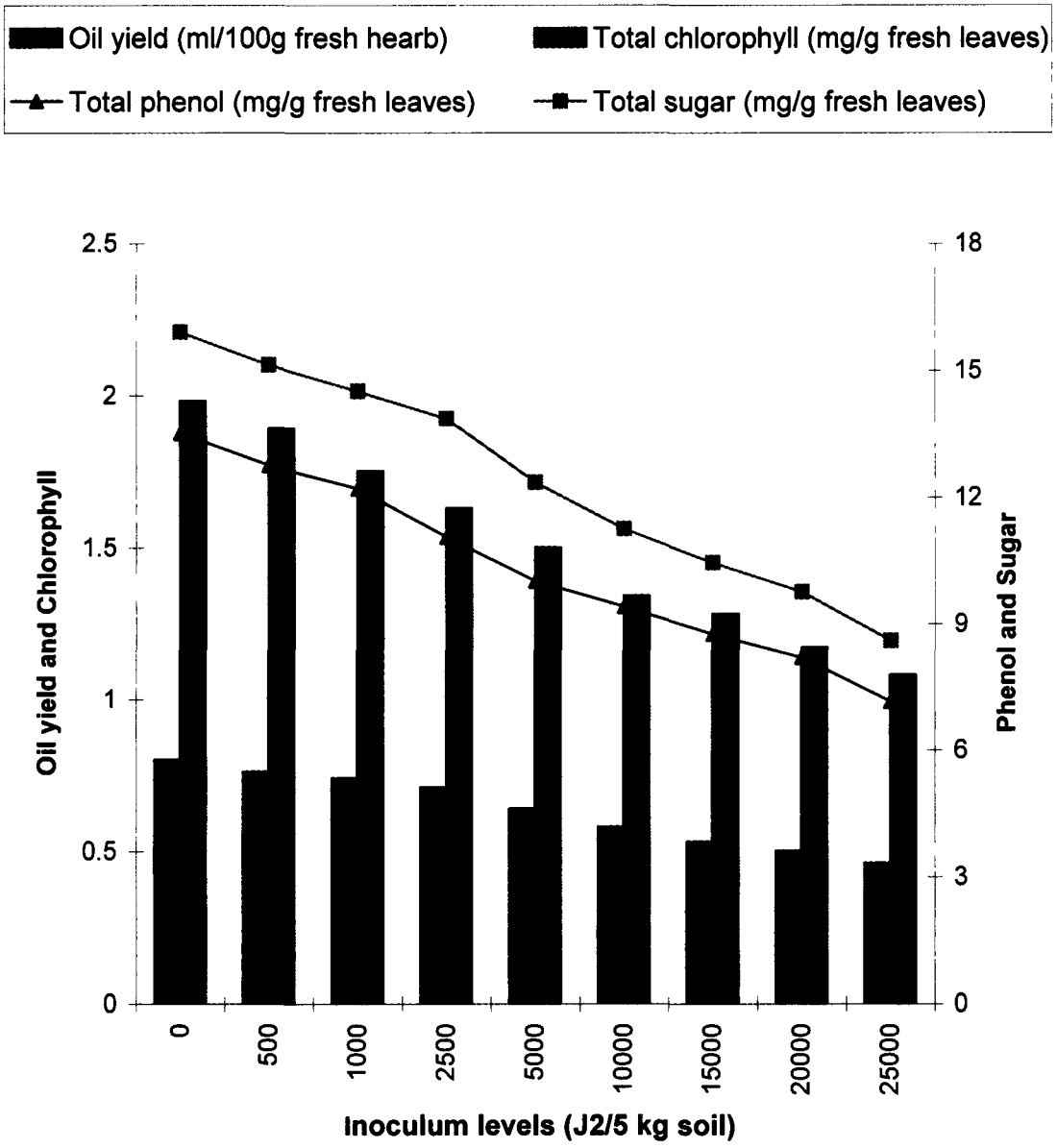


Fig. 4: Effect of different inoculum levels of *M. incognita* on oil yield and biochemical changes in plants of *M. arvensis* cv. Gomti.

Inoculum levels of *M. incognita* on *M. arvensis* cv. Gomti influenced the total content of chlorophyll, total sugar and total phenol and showed a decreasing trend with increasing initial nematode population levels. The observations recorded in the present investigations on the deleterious effect of chlorophyll content in leaves of *M. arvensis* cv. Gomti inoculated with different initial inoculum levels of *M. incognita*, have close similarity with the earlier reports (Melakeberhan *et al.*, 1985, 1986; Haseeb *et al.*, 1990, 1993, Pandey *et al.*, 1992).

The results of the present study indicated that total phenol in the leaves of *M. arvensis* cv. Gomti inoculated with *M. incognita* showed a decreasing trend in total phenol with increasing nematode population. Butool, 1993; Haseeb and Shukla, 1994, 1995, 1996 reported similar findings. The decrease in phenolic levels in leaves as compared to controls may be correlated with the reduced metabolic activity of the plant due to *M. incognita* infection, which may cause discontinuity of conducting vessels (Shafiee and Jenkins, 1963; Bird, 1974; Meon *et al.*, 1978; Haseeb, 1983), inhibit the transport of growth hormones (Brueske and Bergeson, 1972; Meon *et al.*, 1978) and reduce the uptake of water and nutrients (Hanaunik and Osborne, 1975; Meon *et al.*, 1978).

The increase in initial inoculum levels of *M. incognita* also showed a corresponding decrease in total sugar content in leaves of *M. arvensis* cv. Gomti. Other workers have also made similar observations (Owens and Specht, 1966; Singh *et al.*, 1978; Butool, 1993). Sugar content in leaves of *M. arvensis* cv. Gomti was probably affected due to dicontinuity of conducting vessels impeded by the deformation of vascular tissues (Bergeson, 1966), or reduced metabolic activity of the plant due to *M. incognita* infection (Bird, 1974; Melakeberhan *et al.*, 1985).

Observations on the studies regarding the effect of initial inoculum densities of *M. incognita* on the growth and yield of *M. arvensis* cv. Gomti indicated that at initial population of 0.1 J2/g soil and its final population 3.6 J2/g soil resulted above 5% reduction. Observation made from survey studies indicated that initial inoculum of 0.1 J2/g soil resulted the final population of

0.42 J2/g soil (on an average of 6 localities), whereas, final population was 3.3 J2/g soil (average of 15 localities) at highest available initial population of 0.5-1.0 J2/g soil in farmers field. To establish the relationship between initial and final populations in field and under controlled conditions, especially in terms of yield losses, several biotic and abiotic factors have to be considered. Under field conditions lower rate of nematode reproduction may be due the presence of several natural enemies, competition with other plant-parasitic nematodes and pathogenic microorganisms and even due to reaction of different varieties of *M. arvensis*. Therefore, on the basis of initial population it is not appropriate to correlate the yield losses. However, on the basis of final nematode population, it is concluded that under field conditions the damage would have been above 5.0% at the highest population of *M. incognita* (3.3 J2/g soil), and the damage might have been increased further due to soil borne fungi.

3. Establishment of pathogenicity of soil-borne fungi isolated from roots and suckers collected during survey

When an organism is found associated with the diseased part of the plant, the following steps of Koch's postulates are taken to verify the hypothesis that the isolated organism is the actual causal pathogen of the disease (Agrios, 2000):

1. The pathogen must be found associated with the disease in all the diseased plants examined.
2. The pathogen must be isolated and grown in pure culture on nutrient media, and its characteristics described (non-obligate parasites), or it must be grown on a susceptible host plant (obligate parasites), and its appearance and effects are recorded.
3. The pathogen from pure culture must be inoculated on healthy plants of the same species or variety on which the disease appears, and it must produce the same disease on the inoculated plants.
4. The pathogen from pure culture must be isolated in pure culture again, and its characteristics must be exactly like those observed in step two.

Thus fungi isolated from roots and suckers of infected plants collected during survey were tested for their pathogenic potential.

REVIEW OF LITERATURE

Pathogenicity is the capacity of pathogen or parasite to cause disease. Thus when organism is found associated with the plant, it is necessary to findout its pathogenic capability or potential.

In England, Ware (1923) observed *R. violacea* forming a violet felt around the under ground parts of Japanese mint and accordingly named the disease as violet felt rot. The same type of rot caused by *R. crocorum* affecting 50-70% of the crop was termed as violet root rot by Wormald (1938).

Japanease mint and spearmint reported to be attacked by *R. solani* causing leaf rot around Nagpur (Sharma and Mahmud, 1951) and Jammu (Ganguli and Pandotra, 1962), respectively.

Sattar and Husain (1978) reported a seveare wilt of *M. arvensis* caused by *F. oxysporum* from the Gangetic plains of U.P. On the under ground plant parts the symptoms of vascular browning in the roots lets spreaded to basal region of the main root and suckers were observed.

Singh *et al.* (1997) observed stolon decay disease, due to *S. sclerotiorum* on *M. arvensis* var. *piperescens.*

MATERIALS AND METHODS

3.1 Production of cultures of *F. pallidoroseum*, *F. solani*, *R. solani*, and *S. sclerotiorum.*

Conical flasks (1 liter capacity), each containing 400 ml potato dextrose broth (PDB), were autoclaved at 1 kg/cm^2 pressure for 30 min. The flasks were allowed to cool to room temperature, afterwards each flask was inoculated separately with 1-cm-diameter PDA disc punched from a sterilized cork borer from the periphery of the actively growing 5-day-old fungal cultures. The flasks

were placed in a BOD incubator maintained at 25 ± 1 °C and fungi were allowed to grow for 7 days.

Cultures of *F. pallidoroseum* and *F. solani* grown on PDB were shaken and number of spores in medium was adjusted to 10^8 spores/ml by adding required amount of sterile distilled water. Twenty ml suspension was taken into a conical flask and diluted with 180 ml of sterile distilled water. Fifty ml of final suspension was poured in each pot.

Mycelial mats of *R. solani* and *S. sclerotiorum* grown in conical flasks on PDB were picked and placed on sterilized blotting paper lined in glass funnel to drain the medium. Twenty-gram mycelium mat was blended with sufficient amount of sterile distilled water in warring blender for 30 sec. at 1200 rpm and final volume was made up to 200 ml with sterile distilled water. The resulting suspension was poured in pots @ 50 ml/pot.

3.2 Transplanting and inoculation

The experiment was carriedout in 15-cm-diameter clay pots contaning 1 kg steamed sterilized soil and farmyard manure (5:1) mixture. Suckers of *M. arvensis* cv. Gomti were transplanted in the same manner as described in 2.3. At 4^{th} leaf stage soil was removed exposing suckers/roots and predetermined amount of fungal suspension was poured over the exposed roots/suckers, afterwards suckers were covered gently with sterilized soil. There were four replicates of each fungus.

3.3 Recording of data

After two weeks plants were uprooted and suckers/roots were washed under tap water. Percent roots/suckers infection by fungus was determined by procedure described in 1.5.4.

RESULTS

Observations of roots and suckers regarding pathogenicity of different fungi (isolated from roots and suckers of *M. arvensis* collected during survey)

indicated that *S. sclerotiorum* caused most severe infection (40%) of roots/suckers of *M. arvensis* cv. Gomti followed by *R. solani* (14%), *F. solani* (9%) and *F. pallidoroseum* (5%), respectively.

All the test fungi were compared with their original cultures after reisolation on potato dextrose agar medium from roots/suckers of *M. arvensis* cv. Gomti and were found same.

DISCUSSION

Pathogenicity test indicated that all the test fungi were pathogenic to *M. arvensis* cv. Gomti. *S. sclerotiorum* showed highest infectivity potential as compared to other fungi. Being most potential, *S. sclerotiorum* was selected as the test pathogen for present studies. This opinion was also confirmed by reports available in literature on mint and other crops (Purdy, 1979; Willets and Wong, 1980; Singh *et al.*, 1997)

4. Effect of different initial inoculum levels of *S. sclerotiorum* on disease development, growth, oil yield and biochemical changes in plants of *M. arvensis* cv. Gomti

The genus *Sclerotinia* belongs to the family Sclerotineaceae of class Ascomycotina. It is polyphagous in nature, widespread and destructive pathogen of various crops (Walker, 1969; Lumsden, 1979; Purdy, 1979; Tu, 1986; Agrios, 2000). *S. sclerotiorum* (Lib.) de Bary has been reported to attack over 360 species of plants belonging to 54 families (Purdy, 1979).

REVIEW OF LITERATURE

In several studies, pathogenicity tests of *S. sclerotiorum* on different host plants revealed considerable variation in its disease severity. Natti (1971) made a study during three seasons for the first occurrence and subsequent development and spread of white mold disease caused by *S. sclerotiorum* in snapbean plantings in 12 fields. He concluded that the epidemics of white mold occurred 8 to 14 days after full bloom irrespective of planting dates and environmental conditions during the blossom period.

Sehgal and Agrawat (1971) confirmed pathogenicity of *S. sclerotiorum* on one-month-old *Foeniculum vulgare* plants by inoculating 8 days old culture. They noted the symptoms as water soaked specks around the stem at ground level, appeared 10 days after inoculation, which gradually enlarged by advancing upward and downwards. The young plants showed drooping and toppled down within 7 days after appearance of initial symptoms. However, the older plants resisted to the attack of pathogen for longer time, but ultimately collapsed.

Watson *et al.* (1974) proved the pathogenicity of *S. sclerotiorum* on *Centaurea diffusa* and *C. maculosa* by inoculating the hyphal suspension of *S. sclerotiorum*. They reported that the typical wilt symptoms appeared after 10 days of inoculation. Basal leaves of mature plants and all leaves of rosettes and seedlings died due to fungal attack. The sclerotia were observed within the root crown of mature plants after three weeks of inoculation.

Abawi and Grogan (1975) observed a positive correlation between the plant age of *P. vulgaris* and percent parasitism by *S. sclerotiorum* inoculum (apothecia). There was an increase in percentage of infected plants with the increase in duration of parasitism by the fungus. The incidence of disease was found to be more severe on the older than the younger plants.

Adams and Tate (1975 and 1976) while studying the effect of inoculum density of *S. sclerotiorum* and soil moisture fluctuations on the incidence of lettuce drop, noted the highest incidence of the diseases in the field when inoculum density ranged between 0-20 sclerotia/100 g soil. However, in green house 250 sclerotia/100 g soil was required for the expression of same level of disease incidence.

Letham *et al.* (1976) studied the influence of different sources of inoculum (mycelium and ascospores) and host crops on the mode of disease development. They carried out their studies on cauliflower and trellis tomatoes in New South Wales and found that the number of apothecia produced under cauliflower was 10 times that produced under trellised tomatoes and only aerial infection of cauliflowers was recorded, whereas, both aerial and basal infections

due to mycelia and ascospores respectively were observed in tomato for disease development.

Dorrell and Huang (1978) studied the effect of sclerotia sown with seeds (1:3), on sclerotinia wilt of sunflower and observed that the wilt symptoms was 7% at anthesis and it was 60% at eight weeks after the initiation of flowering. The losses in seed yield were 98, 70 and 12% when plants were infected one week, three to four weeks and 8 weeks, respectively after anthesis. Sixty-four percent reduction in seed weight of infected plant was observed after one week of anthesis. The oil content was also reduced upto 30% in plants inoculated two weeks after anthesis.

Huang (1983), while studying the pathogenic potential of two different strains of *S. sclerotiorum* (tan sclerotial and black sclerotial) on the disease incidence in sunflower, found that the seedlings of sunflower were more infected and killed by tan sclerotial strain than black sclerotial strains. He also noted that tan sclerotia survived poorly in soil under green house and field conditions.

Sharma (1985) studied the effect of various environmental factors on the development of white rot of pea due to *S. sclerotiorum* and concluded that the low temperature and high moisture on the plant surface favoured the disease initiation in the post flowering stages of the crop.

Ramsey and Lorbeer (1986) reported that *Botrytis* (*Sclerotinia*) *squamosa* infected a higher percentage of onion florets than either *B. cinerea* or *B. allii,* when umbels with all the florets unopen or umbels with two-thirds of the florets open were inoculated in a dew chamber. They also observed that *S. squamosa* infected the florets more than *B. cinerea* or *B. allii* at all concentrations of inoculum tested in the study.

Tu (1987) reported that the epidemiology of *S. sclerotiorum* in navy bean depends on several factors such as, soil inoculum, soil moisture, rainfall, cultivar susceptibility, and row width and plant density. He found that the fields with high inoculum density and soil moisture showed high disease incidence. However, the reduction in row width and increase in plant density also increased the disease incidence. It was further observed that in a susceptible cultivar

(Fleetwood), the percentage of infected plants increased from 0 to 100% in 4 weeks in 80 cm row planting, while in a tolerant cultivar (ExRico 23), it progressed from 0 to 35%.

Grau (1988) estimated a yield loss of 230 lb/acre at 10 percent incidence of Sclerotinia stem rot in soybean crop and observed a linear relationship between yield and disease severity.

Gulya *et al.* (1989) studied the effect of head rot caused by *S. sclerotiorum* on seed yield, seed weight and oil content of sunflower in 25 different fields of North Dakota, Canada. In "oil seed sunflower", the head rot was found to be responsible for 31% reduction in yield, 10% in seed weight and 2% in oil content, whereas, in "confection sunflower", the yield reduction per head and seed weight was 36 and 11% respectively.

Sattar and Alam (1993), while studying the pathogenicity of *S. sclerotiorum* on *Trachyspermum ammi*, reported 80% mortality of plants as a result of infection by the pathogen.

Sala *et al.* (1994) determined the effect of Sclerotinia mid-stalk rot on various components of yield as well on oil content of sunflower. *S. sclerotiorum* was found to inflict an average reduction of 35% in seed yield, 24% fewer seeds/head, a 15% decrease in 1000 seed weight and lower oil content in 6 different sunflower hybrids as compared with healthy plants.

Sattar *et al.* (1995) made pathogenicity tests under glass house conditions using different isolates of *S. sclerotiorum* on various hosts i.e. Egyptian henbane, black henbane, French basil, Sowa and Damask rose. The experimental results showed that all host plants were highly susceptible to infection exhibiting visible symptoms of the disease on the tested plants.

Sala *et al.* (1996) studied yield losses of sunflower due to *S. sclerotiorum* head rot under field conditions in Argentina. They found different levels of disease intensity (percentage of diseased plants) in 5 commercial hybrids under different environmental conditions. They reported that the highly positive correlations occurred between percentage of diseased plants and reduction in seed yield ($r = 0.76$) and between increased dackage ($r = 0.67$) and oil acidity

levels (r = 0.58). Yield reduction estimates varied among the hybrids, suggesting that they varied genetically in their disease tolerance.

Aggarwal *et al.* (1997), while investigating the effects of *S. sclerotiorum* infection on oil content and quality in low erucic acid cultivars of *Brasica napus* cv. Culture-2 and *B. compestris* cv. Tobin, observed that the oil content and quality decreased in diseased plants of both cultivars.

Sharma *et al.* (2002) evaluated various species/varieties of *Brassica* for resistance against *S. sclerotiorum* and found that in *B. juncea* group var. KLM-1359 had the least (around 30%) plant mortality as compared to var. Varuna, where mortality was observed to be more than 80%. Amongst other *Brassica* spp., *B. napus* was found to be least susceptible followed by *B. carinata*. While in *B. compestris, B. tournifortii, B. chinensis, Eruca sativa* and *Raphanus sativus,* the plant mortality exceeded 70%.

Biochemical changes in plants

The fungal pathogens like other pathogenic microorganisms usually cause disease in plants by disturbing the metabolism of plant cells, which affects various physiological and biochemical functions of the host plant (Agrios, 2000).

Inman (1962) studied the disease development, disease intensity and carbohydrate levels in rusted *P. vulgaris* var. Great Northern No. 59 due to *Uromyces phaseoli* var. *typica* race 3. The carbohydrate levels in primary leaves were found to be dependent on stage of disease development and intensity of infection. He also noted that the increased concentration of sucrose and slightly decreased trend in reducing sugar levels with the increase in infection. Sugar levels declined with the advent of sporulation to points considerably below those in healthy tissues. However, the higher infection intensity resulted in more abrupt decline.

Vir and Grewal (1974) studied the changes in phenolic content of chickpea due to infection of *Ascochyta rabiei*. No statistical difference was observed in phenolic contents of resistant and susceptible varieties prior to inoculation. However, after inoculation there was significant increase in phenol content in both varieties. Phenol content was increased with the increase in

sampling intervals but increase was significantly more in inoculated resistant variety than the susceptible one.

Sharma and Wahab (1975) made an attempt to investigate the changes in sugars and protein content of *Luffa cylindrica* fruits infected with *P. aphanidermatum.* In this study, they observed that the level of soluble free sugars in diseased fruit varies with disease development. It was also recorded that there was a continuous decline in soluble free sugars upto 16 h after inoculation followed by a noticeable rise at 20 h beyond which the level remains almost constant. A marked decrease in their level was noted in healthy fruits between 4 to 12 h of incubation.

Garg and Mandahar (1976) studied the changes in physiology of *Abelmoschus esculentus* leaves infected with *Erysiphe polygoni.* They found that starch content in infected leaves decreased progressively with disease development, but reducing sugars increased in powdery mildew-infected leaves as compared to control. Mukhopadhyay and Nandi (1976) determined the carbohydrate content of jute plants infected with *M. phaseolina.* The total carbohydrate level of an infected plant was noticeably less as compared to healthy plant of the same age.

Singh and Bedi (1976) estimated phenolic and sugar constituents of gram cultivars resistant and susceptible to *Operculella padwickii.* They found that resistant cultivar had more total phenols, flavonols and tannins as compared to susceptible ones. As a result of fungal infection, total phenols, flavonols and tannins increased in both the cultivars but increase in phenolics was more in resistant cultivar G-543 than in susceptible cultivar K-4. Sugars were higher in the healthy tissues of the susceptible cultivar than the resistant ones.

Prasad *et al.* (1976) studied the biochemical changes in the leaves of safflower infected with *Puccinia carthami.* They reported the reduction in chlorophyll and sugar contents of the infected leaves as compared to healthy leaves.

Upadhyay and Dwivedi (1979) determined the biochemical changes in the leaf of *Eucalyptus globus* infected with *Pestalotiopsis funerea*. Results showed that sugars, organic matter, chlorophyll a, b and energy level were decreased in plants inoculated with *P. funerea* as compared to healthy leaves. Chlorophyll content was also found to be very low in lesion as compared to halo and fresh regions of the infected leaves.

Lily and Ramadasan (1979) determined the changes in phenolic content of coconut leaf in relation to the development of leaf rot caused by *Bipolaris halodes*. The observations exhibited a significant increase in the total phenol content in the inoculated leaves as compared to healthy leaves.

Shree and Reddy (1986) carried out a comparative study in relation to different biochemical constituents in the healthy and *Exserohilum turcicum* infected leaves of susceptible and resistant cultivars of sorghum at different stages of crop growth and infection. Healthy resistant cultivars contained comparatively high amount of inherent total phenols, reducing sugars, free amino acids and proteins at different growth stages as compared to susceptible cultivars. In the infected resistant cultivars, these constituents accumulated at higher levels as compared to the inoculated susceptible cultivars.

Babu and Reddy (1989) studied the changes in sugar content of lemon fruit infected with *Colletotrichum gloeosporioides* and *Syncephalastrum racemosum*. They reported that during advanced stages of infection there was drastic reduction in the concentration of sugars. Prasad *et al.* (1989) reported reduction in starch, sugars (reducing, non-reducing and total), chlorophyll (a, b and total), carotene, and xanthophyll contents and an increase in the content of Glucose 1-phosphate and glucose 6-phosphate in the coriander leaves infected with *Protomyces macrosporus*.

Gupta *et al.* (1990) studied the reduction in total phenol content in the leaves of mustard cultivars infected with *Alternaria brassicae* and *A. brassicola*. The reduction was found to be higher in infected susceptible cultivars as compared to infected tolerant cultivars.

Malhotra (1991) observed the changes in phenolic contents in resistant and susceptible genotypes of tomato infected with *F. oxysporum* f. sp. *lycopersici*. He reported that after infection there was a significant increase in phenolic contents in both resistant and susceptible genotypes. However, the increase was more in resistant than in susceptible varieties.

Sehtiya *et al.* (1992), while studying the effect of *C. falcatum* on the sucrose content of susceptible and resistant sugarcane cultivars, noticed a reduction of 40% in the inoculated susceptible sugarcanes as compared to control, whereas no reduction in sucrose content was observed in inoculated resistant cultivars.

Jain *et al.* (1994) observed the changes in total soluble sugar, starch contents and α-amylase activity in coriander stem infected with *P. macrosporus*. They concluded that an increase in total soluble sugars and α-amylase activity in galled tissues, both *in vivo* and *in vitro* as compared to normal tissues. However, normal tissues showed higher starch contents than the galled tissues.

Kumar and Singh (1996) studied the changes in chlorophyll and sugar content of sunflower leaves in relation to Alternaria blight development and reported that there was a drastic reduction in chlorophyll a and b in the diseased leaves. However, higher sugar content was found in healthy leaves, whereas, considerable reduction in sugar content was noticed after 40 and 70 days of inoculation in necrotic tissues.

Dharmadhikari and Jite (1996) studied the changes in the levels of total sugars and ascorbic acid in *Acacia* plants infected with *Hapalophragmiopsis ponderosa*. The sucrose and ascorbic acid content was decreased in the galled tissues, while, concentration of fructose was increased to a marked extent as compared to healthy tissues.

Sindhan *et al.* (1996) made an attempt to study the changes in biochemical constituents in presence of *Urocystis agrophyri* on resistant and susceptible cultivars/lines of wheat and triticale and observed reduction in total sugar, total phenol and orthodihydric phenols concentration in infected leaves of

all varieties as compared to healthy ones. However, the depletion was more pronounced in susceptible varieties.

Kumar *et al.* (1998) investigated the effect of sclerotinia head rot on some biochemical constituents of sunflower seeds and noticed a drastic decrease in total sugar, phenol and oil contents of seeds due to *S. sclerotiorum.*

Chandrasekaran *et al.* (2000) made a study on the changes of phenolic and oxidising enzymes due to *C. truncatum* in resistant and susceptible cultivars of soybean and concluded that the total phenolic content and Ortho-dihydroxyphenol was found to be significantly higher in the resistant cultivar (both healthy and inoculated) as compared to susceptible one.

MATERIALS AND METHODS

4.1 Maintenance and production of culture of *S. sclerotiorum*

Conical flasks (1 liter capacity), each containing 400 ml potato dextrose broth (PDB), were autoclaved at 1 kg/cm^2 pressure for 30 min. The flasks were allowed to cool to room temperature, afterwards each flask was inoculated with 1-cm-diameter PDA disc punched from a sterilized cork borer from the periphery of the actively growing 5-day-old cultures of *S. sclerotiorum.* The flasks were kept in a BOD incubator maintained at 25 ± 1 °C and mycelial mat allowed to grow for 7 days.

4.2 Transplanting and inoculation

To examine the effect of different initial inoculum levels of *S. sclerotiorum,* suckers of *M. arvensis* cv. Gomti were transplanted in the same manner as described in 2.3. At 4th leaf stage soil was removed exposing suckers and roots of *M. arvensis* cv. Gomti and predetermined amount (0, 1, 3, 6, 9, 12 g) of *S. sclerotiorum* mycelium in aqueous suspension was poured over the exposed roots/suckers, afterwards suckers were covered gently with sterilized soil. There were five replicates of each treatment. The experiment was laidout as a completely randomized block design.

4.3 Recording of data

Recording of data regarding shoot height, roots/suckers-shoot fresh and dry weight was done as described in 2.4.1, and determination of percent roots/suckers infection, estimation of chlorophyll (a, b and total), total sugar, total phenol and oil yield was done in the same manner as described in 1.4 and 2.4.3-2.4.6.

RESULTS

As initial inoculum levels of *S. sclerotiorum* increased, there was a corresponding decrease in shoot height, plant fresh and dry weights (Table 9; Fig. 5; Plate 6). The maximum reduction in the shoot height, shoot-roots/suckers fresh weight and shoot-roots/suckers dry weights (30.4, 39.8, 43.6, 40.3 and 42.9%), respectively, was observed at the highest initial inoculum level of 12 g fungal mycelium/5 kg soil as compared to uninoculated control. Analyses of data indicated that effects of all the inoculum levels on all the test parameters were highly significant ($P \leq 0.01$).

The infection of roots and suckers due to *S. sclerotiorum* increased with increasing initial inoculum levels. At the lowest Pi (1.0 g mycelium/5 kg soil), infection was observed 18.0% and at the highest Pi (12 g mycelium/5 kg soil), it was 80.2%. Significant ($P \leq 0.05$) differences were observed in the extent of infection among all the corresponding inoculum levels (Table 9; Fig. 5).

Significant ($P \leq 0.01$) reduction in oil yield, chlorophyll a, chlorophyll b, total chlorophyll, total phenol and total sugar content of *M. arvensis* plants was observed at the lowest inoculum level as compared to uninoculated control. Analyses of data indicated that differences in all above mentioned test parameters were significant ($P \leq 0.01$) among all the initial inoculum levels. Maximum reduction in oil yield, chlorophyll a, chlorophyll b, total chlorophyll, total phenol and total sugar 28.9, 31.3, 32.1, 31.4, 34.8 and 31.6%, respectively, was observed at the highest inoculum level as compared to uninoculated control (Table 10; Fig. 6).

Table 9: Effect of different initial inoculum levels of *Sclerotinia sclerotiorum* (g mycelium/5 kg soil) on disease development and plant growth of *Mentha arvensis* cv. Gomti.[a]

Initial inoculum levels	Shoot height (cm)	Plant fresh weight (g)			Plant dry weight (g)			[b]Roots & suckers infection
		Shoot	Roots & suckers	Total	Shoot	Roots & suckers	Total	
0.0	76.2	130.5	122.2	252.7	31.0	23.5	54.5	0.0
1.0	72.0	119.2	109.1	228.3	28.2	21.0	49.2	18.00
	(5.5)[c]	(8.6)	(10.7)	(9.6)	(9.0)	(10.6)	(9.7)	
3.0	68.5	105.7	96.5	202.2	25.0	18.5	43.5	40.00
	(8.9)	(19.0)	(21.0)	(20.0)	(19.3)	(21.3)	(20.2)	
6.0	63.2	93.5	82.6	176.1	22.0	15.7	37.7	65.00
	(17.1)	(28.3)	(32.4)	(30.3)	(29.0)	(33.2)	(30.8)	
9.0	59.0	85.4	75.5	160.9	20.1	14.5	34.6	72.50
	(22.6)	(34.5)	(38.2)	(36.3)	(35.2)	(38.3)	(36.5)	
12.0	53.0	78.5	68.9	147.4	18.5	13.4	31.9	80.25
	(30.4)	(39.8)	(43.6)	(41.7)	(40.3)	(42.9)	(41.5)	
L.S.D. $_{0.05}$	1.9	3.8	4.0	5.2	1.1	1.0	2.7	2.8
L.S.D. $_{0.01}$	2.5	5.2	5.4	7.2	1.5	1.4	3.6	4.0

[a]Each value is an average of five replicates.
[b]Percent roots and suckers infection by *S. sclerotiorum*.
[c]Figures in parantheses are percent reduction over uninoculated control.

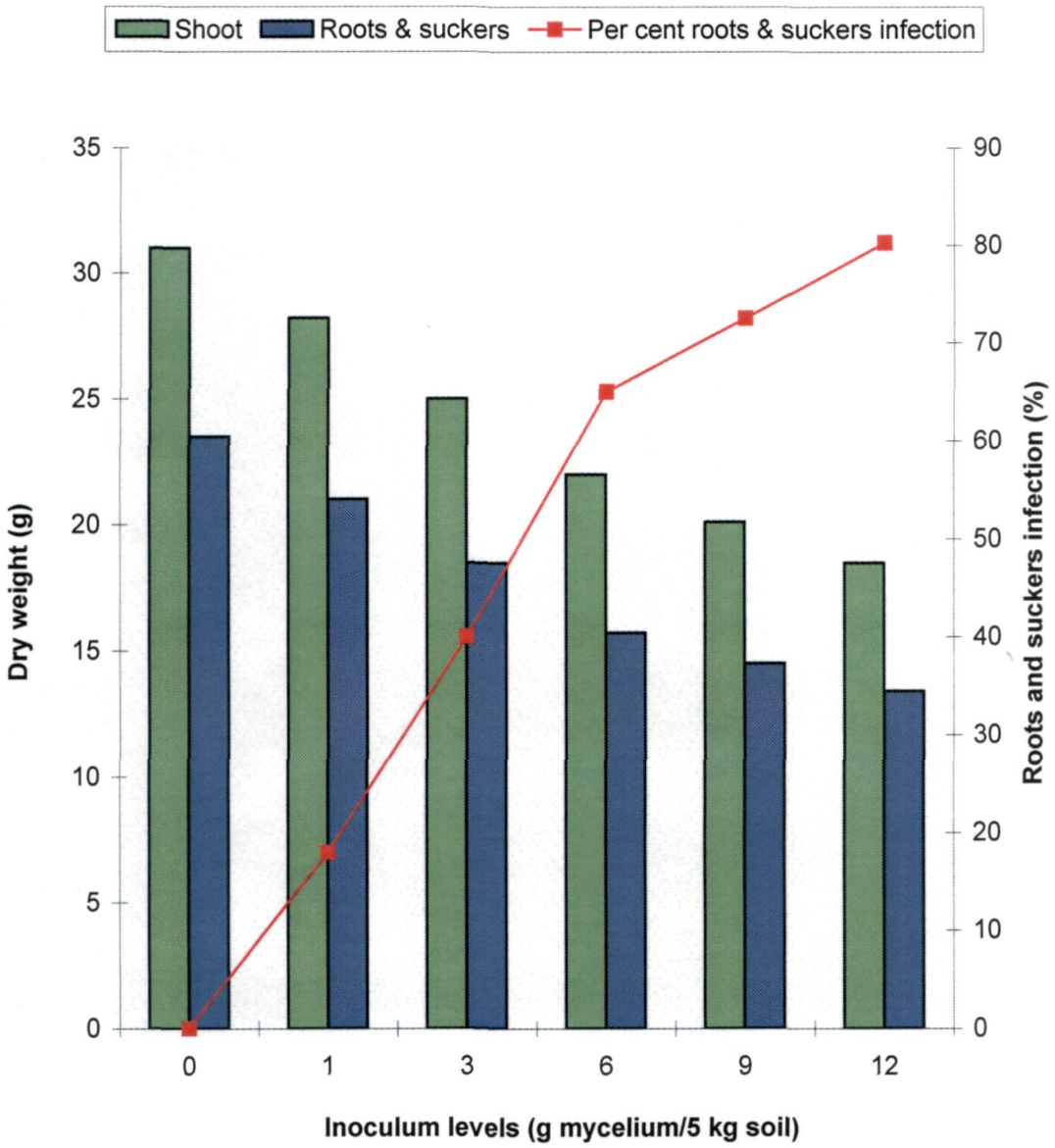


Fig. 5: Effect of different initial inoculum levels of *S. sclerotiorum* on disease development and growth of *M. arvensis* cv. Gomti

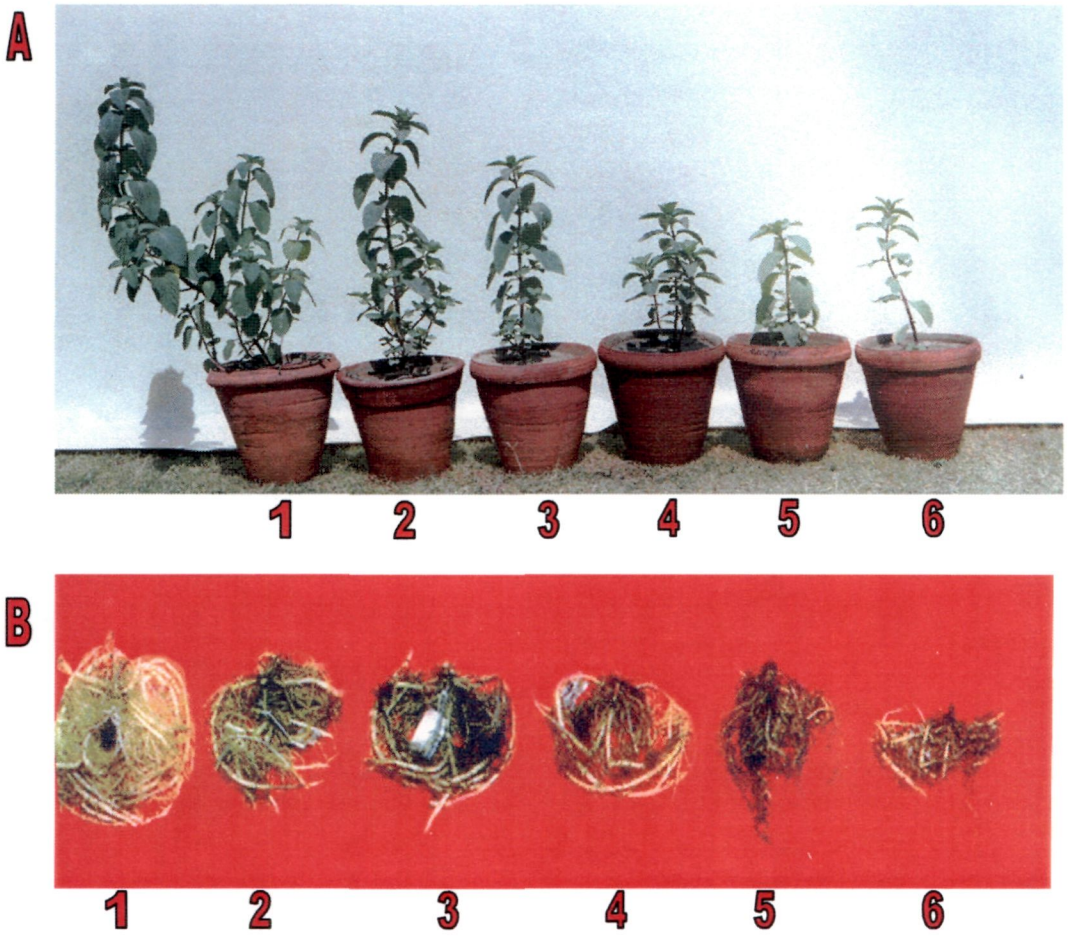


Plate 6: Effect of different inoculum levels of *S. sclerotiorum* on the aerial growth (A) and on roots and suckers development (B) of *M. arvensis* cv. Gomti.

1 = Uninoculated control
2 = 1 g mycelium/pot
3 = 3 g mycelium/pot
4 = 6 g mycelium/pot
5 = 9 g mycelium/pot
6 = 12 g mycelium/pot

Table 10: Effect of different initial inoculum levels of *Sclerotinia sclerotiorum* (g mycelium/5 kg soil) on oil yield and biochemical changes in plants of *Mentha arvensis* cv. Gomti.[a]

Initial inoculum levels	Oil yield (ml/ 100 g fresh herb)	Chlorophyll content (mg/g fresh leaves)			Total phenol (mg/g fresh leaves)	Total sugar (mg/g fresh leaves)
		Chl a	Chl b	Total chl		
0.0	0.76	1.15	0.53	1.69	12.90	15.50
1.0	0.74 (2.60)[b]	1.10 (3.48)	0.51 (3.77)	1.63 (3.55)	12.25 (5.04)	14.75 (4.84)
3.0	0.69 (9.10)	1.07 (6.96)	0.47 (7.55)	1.55 (8.28)	11.60 (10.08)	14.00 (9.68)
6.0	0.65 (14.47)	0.97 (15.65)	0.45 (15.09)	1.43 (15.38)	10.50 (18.60)	12.75 (17.74)
9.0	0.58 (23.68)	0.90 (21.74)	0.40 (24.53)	1.31 (22.48)	9.25 (28.29)	11.20 (27.74)
12.0	0.54 (28.94)	0.79 (31.30)	0.36 (32.07)	1.16 (31.36)	8.40 (34.88)	10.60 (31.61)
L.S.D. $_{0.05}$	0.02	0.02	0.01	0.03	0.19	0.22
L.S.D. $_{0.01}$	0.03	0.03	0.02	0.04	0.27	0.30

[a]Each value is an average of five replicates.
[b]Figures in parentheses are percent reduction over uninoculated control.

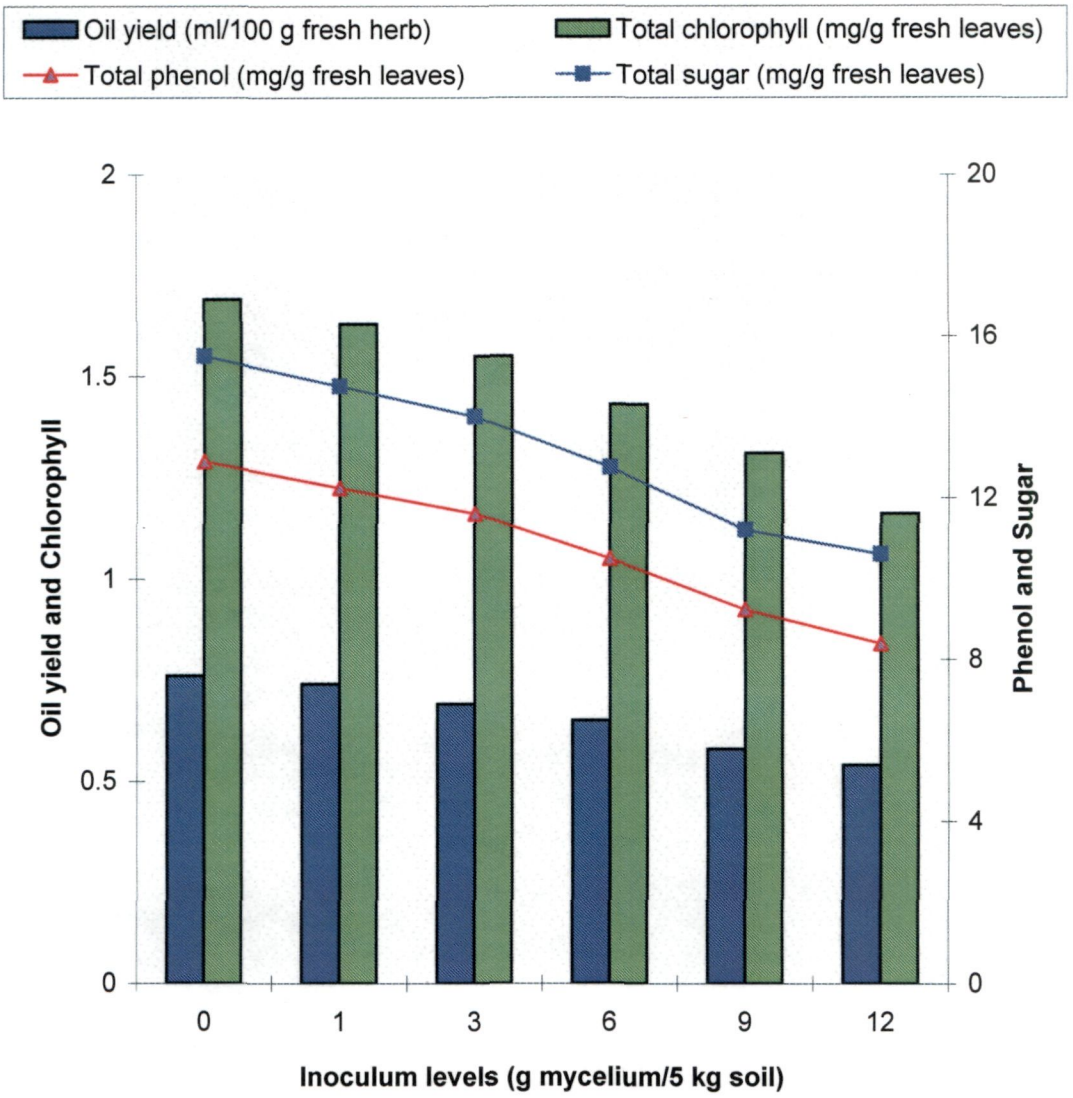


Fig. 6: Effect of different initial inoculum levels of *S. sclerotiorum* on oil yield and biochemical changes in plants of *M. arvensis* cv. Gomti.

DISCUSSION

Similar to the observations recorded in pathogenicity tests with *M. incognita*, the increasing inoculum levels of *S. sclerotiorum* also resulted a gradual increase in extent of reduction in shoot height, shoot and root+sucker fresh and dry weights, oil yield, chlorophyll, total sugar and total phenol content in leaves and root/sucker infection due to fungus. Grau (1998) observed a linear relationship in yield and severity of stem rot on soybean due to *Sclerotinia* spp. In another study, Mehrotra (1973) observed that an increase in inoculum potential of wilt fungus resulted in increased severity of wilt of lentil. Warren (1975) also reported a linear relationship between inoculum concentration, disease severity, and seedling infection due to *R. solani* with lima bean.

Biochemical resistance or susceptibility in plants against any disease mainly depends on pre-existing, preformed or induced substances by the pathogen. Despite wide prevalence of Sclerotinia diseases, meager information is available on the changes in biochemical constituents of the host plant (Willets and Wong, 1980).

In the present study, the results showed a decrease in chlorophyll content in the leaves of *M. arvensis* cv. Gomti, when inoculated with the increasing inoculum levels of *S. sclerotiorum*. The reduction in chlorophyll content might be due to metabolic disruption occurred due to the reduction in protein nitrogen content (Doby, 1965; Padmanabhan *et al.*, 1974).

There was a decrease in total phenol of the leaves of *M. arvensis* cv. Gomti with increase in initial inoculum of *S. sclerotiorum*. Kumar *et al.* (1998) also reported reduction in total phenol content in the seeds of sunflower infected with *S. sclerotiorum*. Similar observations were also recorded in mung beans due to *R. solani* (Arora, 1983) and in wheat due to *Urocystis agropyri* (Sindhan *et al.*, 1996). However, in some instances the phenolic content was increased after infections (Lily and Ramadasan, 1979; Gangopadhyay and Lal, 1986; Malhotra, 1991). In general, the phenolic content has a direct relationship with host resistance against pathogens. An interaction between the host and pathogen may

trigger the production of total phenols and lack of such capacity in the susceptible plants may be responsible for successful establishment of the pathogen (Shree and Reddy, 1986).

Increased reduction of total sugar was also recorded in the leaves of *M. arvensis* cv. Gomti, with increasing inoculum levels of the fungus. Kumar *et al.* (1998) also observed similar results in seeds of sunflower infected with *S. sclerotiorum.* The reduction in total sugar might have occurred because of altered rates of synthetic and respiratory activity due to infection by pathogen (Chopra and Jhooty, 1974).

All the test initial inoculum levels of *S. sclerotiorum* caused significant damage to *M. arvensis* cv. Gomti. The prediction of losses expected from a particular inoculum level of *S. sclerotirum* in soil can be utilized in developing management strategies.

Chapter 4

Interaction

5. Effect of *M. incognita* and *S. sclerotiorum* alone or in combined inoculation on disease development, nematode multiplication, growth, oil yield and biochemical changes in plants of *M. arvensis* cv. Gomti

Plant-parasitic nematodes are pathogens in their own right and are capable of producing recognizable disease symptoms on an appropriate susceptible host. Most of the diseases caused by nematodes are debilitating. However, when they interact with other pathogenic organisms the disease picture drastically altered. It changes from debilitating to annihilating (Powell, 1963, 1971, 1979; Bergeson, 1972). The varieties bred resistant against fungal attack become susceptible in the presence of a nematode (Atkinson, 1892; Neal, 1954; Melendez and Powell, 1965, 1967; Porter and Powell, 1967; Sumner and Johnson, 1973; Hasan, 1985). Soil inhabiting fungi such as *Pythium, Curvularia, Botrytis, Aspergillus, Penicillium* and *Trichoderma* species, which are normally not pathogenic on healthy tobacco roots, become pathogenic on roots infected by root-knot nematode (Powell *et al.*, 1971).

REVIEW OF LITERATURE

The literature on nematode fungal interaction has been reviewed by Pitcher (1963, 1965), Miller (1965), Powell (1971, 1979), Bergeson (1972), Norton (1978), Lamberti and Taylor (1979), Haseeb (1983), Sikora (1984), Webster (1985), Mai and Abawi (1987), Sasser (1989), Luc *et al.* (1993), Khan (1993), Evans and Haydock (1993), Back *et al.* (2002).

Atkinson (1892) for the first time reported that the infection by root-knot nematodes considerably increased the incidence and severity of Fusarium wilt in cotton.

Jenkins and Coursen (1957) reported that the tomato var. Chesapake resistant to *Fusarium* become susceptible in the presence of root-knot nematodes. Further more, when *M. hapla* was combined with the fungus, 60% of the plants wilted, whereas, *M. incognita acrita* promoted wilt in 100% of the

plants. Reynolds and Hanson (1957) reported that post-emergence damping off in cotton, caused by *R. solani,* become severe in presence of *M. incognita acrita.*

McGuire *et al.* (1958) observed that wilt incidence on alfalfa was increased when *F. oxysporum* f. sp. *vasinfectum* was combined either of *M. hapla, M. javanica, M. incognita* and *M. arenaria.* Apt and Koike (1962) reported that *Pythium graminicola* and *M. incognita acrita* produced ill effect on sugarcane. However, the ill effect was restricted to top growth and not to root growth.

Minton and Minton (1963) studied the effect of root-knot-Fusarium complex on cotton. Studies revealed that the fungus colonized the giant cells of both the resistant and susceptible plants. As a result of which the giant cells become devoid of protoplasm. Bowman and Bloom (1966) demonstrated indirect relationship of *M. incognita* to the breaking of resistance to Fusarium wilt in tomato, which appeared to change the physiology of the entire plant thereby making it more susceptible to Fusarium wilt.

Porter and Powell (1967) observed that *M. incognita* or *M. javanica* or *M. arenaria* predisposed tobacco plants to the attack of *F. oxysporum* f. sp. *nicotianae*. However, the damage was more severe when nematode infection preceded the fungus by 2 to 4 weeks.

Powell *et al.* (1971) tested flue-cured tobacco cultivar C-316 susceptible to *M. incognita* against species of the soil-inhabiting fungi viz. *Pythium, Curvularia, Botrytis, Aspergillus, Penicillium* and *Trichoderma.* The roots showed symptoms of necrosis when tobacco plants were subjected to the attack of *M. incognita* in combination with any one of these fungi. Necrosis was especially severe in treatments in which nematodes preceded the fungi by several weeks. None of the fungi induced disease unless *M. incognita* was present.

Golden and Van Gundy (1975) reported that in okra and tomato plants, root decay was more in plants when they were inoculated by *R. solani* 4-5 weeks after the inoculation of *M. incognita*. It was observed that *R. solani* colonized

giant cells and xylem cells of the roots. Fungal sclerotia were formed only on galled tissues. The fungus penetrated either directly or through ruptures in the root created by the mature female nematode.

Carter *et al.* (1977) reported that the reduction in shoot dry weight of the susceptible cultivar of tomato "person improved" was 9, 16, and 47 percent when plants were inoculated with *M. incognita* and *F. oxysporum* f. sp. *lycopersici* alone or together, respectively.

Sharma and Gill (1979) studied the interrelationships between *M. incognita* and *R. solani* on potato. The results revealed that both *M. incognita* and *R. solani* reduced shoot weight significantly in the plants inoculated with any one pathogen, and their combined effects were observed to be almost equal to their individual effect except in treatment wherein fungus preceded nematodes. The total nematode multiplication per gram of root weight was highest where nematodes were inoculated singly and was significantly reduced in all the treatments in which fungus was present. Similar results were also observed by Reddy *et al.* (1979) on French bean.

Chhabra and Sharma (1981) determined the combined effect of *M. incognita* and *R. bataticola* on pre-emergence damping-off of okra and brinjal. They found that germination and growth of okra and eggplant were reduced more when both *M. incognita* and *R. bataticola* were present together than when either pathogen was present alone.

Khan and Muller (1982) studied the interaction between *R. solani* and *M. hapla* on radish in gnotobiotic culture in petri plates. They observed that prior infection of roots by nematode favoured the colonizing capability of the fungus. Galls were preferred by the fungus and mycelium accumulated over them. Vigorous mycelial growth and abundant sclerotial formation was observed on galls. Non- galled portions did not show sclerotia formation, but more hyphal growth and penetration was observed in contrast to the roots inoculated with fungus alone.

Chahal and Chhabra (1984) reported that the simultaneous inoculation of *M. incognita* and *R. solani* on tomato plants caused significant reduction in shoot weight and length and root weight in comparison to either of the pathogen alone. The maximum number of galls and nematode population was observed in plants inoculated with *M. incognita* three weeks prior to *R. solani.*

Hasan (1985) investigated the role of *R. solani* and *P. aphanidermatum* in the breakdown of resistance in chilli cvs. "Jowala" and "Longthin Faizabadi". The gall index and number of egg masses and eggs produced on each root system increased significantly on the two cultivars when the plants were inoculated with either of the fungi and nematode simultaneously or prior to nematode.

Al-Hazmi (1986) carried out an experiment in a glass house to study the interaction of *M. incognita* and *M. phaseolina* in a root-rot disease complex of French bean. He reported that the severity of Macrophomina root-rot of French bean increased when nematode was inoculated prior to fungus, whereas nematode infection and reproduction were reduced when the fungus was introduced prior to nematode.

Zaidi and Tiyagi (1989) studied the interaction between *M. incognita* and *F. solani* on chilli under pot conditions. The maximum reduction in plant growth was observed when *M. incognita* and *F. solani* were inoculated simultaneously, followed by 10 days prior inoculation of nematode than fungus and inoculation of *F. solani* 10 days prior to *M. incognita* respectively.

Sitaramaiah and Devi (1990) observed significant reduction in plant growth of betelvine in plants inoculated with *M. incognita* and *S. rolfsii* simultaneously as compared to plants inoculated with *M. incognita* and *S. rolfsii* alone. Maximum root-knot index (4.7) was observed in plants inoculated with *M. incognita* alone. Significantly lower root-knot nematode population was recorded in combined inoculation of plants with *M. incognita* and *S. rolfsii.*

Khafagi *et al.* (1992) inoculated nine strawberry cultivars with *M. incognita* or *M. javanica* alone or in combination with *F. oxysporum* f. sp.

fragariae. Highest reduction in plant dry weight was observed in plants inoculated with *M. javanica* and *F. oxysporum* f. sp. *fragariae* simultaneously. Nematode has enhanced the development and severity of wilt disease.

Rao and Krishnappa (1994) reported that the inoculation with *M. incognita* and *F. oxysporum* f. sp. *ciceri* adversely affected the growth of chickpea. The reduction in plant growth, root-knot development and wilt incidence were more in plants inoculated with nematode and fungus simultaneously than in plants inoculated with nematode or fungus alone. Highest wilt incidence (66.67%) was observed in plants inoculated with 2 juveniles/g soil and 25 g fungus inoculum 500 g soil.

Kassab and Ali (1995) studied the effect of *M. incognita* and *F. oxysporum* f. sp. *lycopersici* on tomato cv. Walter, resistant to fungus. They observed that pre-inoculation with nematodes allowed fungi to colonize the root more extensively than in plants inoculated with both the pathogens simultaneously or when the fungus preceded nematodes. Marley and Hillocks (1996) also reported the similar results on resistant cultivars of pigeonpea, in plants inoculated with *M. incognita* or *M. javanica* prior to *F. udum.*

Walker (1997) studied the effect of *Meloidogyne* spp. and *R. solani* on the growth of grapevine rooting. They reported that root rotting was higher in grapevines inoculated simultaneously with *M. incognita* and *R. solani* and the highest rotting was noted in grapevine inoculated with nematode prior to fungus. Shoot weights were found to be lower when vines were inoculated with the nematode 13 days before the fungus, compared with the inoculation of both the nematode and fungus simultaneously. Makhnotra *et al.* (1997) reported that the incidence of rhizome rot of ginger was higher in plants inoculated with *M. incognita* and *F. oxysporum* simultaneously than either of the two pathogens inoculated alone.

Perveen *et al.* (1999a, 1999b) determined the interrelationship between different initial inoculum densities of *M. incognita* and *F. udum*, and their sequential or simultaneous inoculation on the growth of *Cajanus cajan* cv. Bahar and Gwaliar-2. Results showed inversely proportional relationship between

initial inoculum densities (Pi) of *M. incognita*, *F. udum* and the plant growth parameters of both cultivars of pigeonpea. While, the relationship between final nematode population/root-knot index/percent root colonization by the fungus and Pi was observed directly proportional. The studies regarding interactive effect of pathogens on pigeonpea cv. Bahar and Gwaliar-2 showed the highest reduction in plant growth parameters in plants inoculated with nematode and fungus simultaneously followed by nematode one week prior to fungus and nematode one week post to fungus respectively.

Walker *et al.* (1999) studied an interaction on cotton involving *M. incognita* and *Thielaviopsis basicola.* They reported that in the presence of both pathogen, there was an increase in cotton seedlings mortality and suppression of early seedlings growth as compared to the either of the pathogen present alone. The reduction in plant growth was observed highest in plants inoculated with both the pathogens simultaneously. Maximum expression of disease was observed in early part of the growing season of the crop in Arkansas.

Bhagwati and Goswami (2000) studied the effect of *M. incognita* and *F. oxysporum* f. sp. *lycopersici* on tomato. The synergistic effect was recorded in treatments with either simultaneous inoculation of the pathogens or where nematode inoculation preceded 10 days to fungus.

Haseeb and Shukla (2000b) reported that the severity of root-knot disease of Japanese mint was increased under field conditions in presence of several soil borne fungi, particularly the species of *Fusarium* and *Rhizoctonia.* Studies carried out under controlled conditions indicated the severe reduction in growth and oil yield at lower initial inoculum densities, whereas, at higher inoculum levels, young plants could not survive.

Singh and Goswami (2001) reported that *M. incognita* enhanced wilting of cowpea cultivar Pusa Komal when inoculated in combination with *F. oxysporum.* Nematode inoculation preceded by fungal inoculation showed maximum expression of the disease followed by the treatment when both the pathogens were inoculated simultaneously. Presence of nematode not only predisposed the host but also shortened the incubation period for disease

expression. Nodulation in roots were affected in plants inoculated with both the pathogens simultaneously or nematode prior to fungus.

Anwar and Khan (2002) reported that the *M. incognita* inoculated prior and after *R. solani* treatment resulted in only slight to moderate inhibition of galling, nodulation and female development. Simultaneous inoculation of fungus and nematode exhibited a linear decrease in final population of nematode in soybean. Highest decrease in grain weight, growth parameters, chlorophyll (a, b and total) and oil contents were observed in simultaneous inoculation of both pathogens followed by nematode prior to fungus, nematode post to fungus and fungus alone respectively.

MATERIALS AND METHODS

5.1 Transplanting and inoculation

Inoculation of *S. sclerotiorum* and *M. incognita* was done according to the following scheme:

(i) Uninoculated control

(ii) 5000 J2 alone

(iii) 5000 J2 + 3 g fungus mycelium simultaneously

(iv) Inoculation of 5000 J2 a week prior to inoculation of 3 g fungus mycelium

(v) Inoculation of 5000 J2 a week after the inoculation of 3 g fungus mycelium

Five replicates were maintained for each treatment. The experiment was laidout as a completely randomized block design. Procedures for the transplanting of suckers, inoculation of nematode and fungus were same as described earlier in 2.3 and 4.2.

5.2 Recording of data

Recording of data regarding plant growth, nematode population, estimation of chlorophyll, total sugar, total phenol and oil yield was done in the same manner as described in 2.4, and root-knot index and percent roots/suckers

infection by the fungus was dertermined as mentioned in 1.3 and 1.4, respectively.

RESULTS

In general, greater suppression of shoot height, fresh and dry shoot-root/sucker weights, oil yield, chlorophyll, sugar and phenol content of leaves was observed in plants inoculated with *M. incognita* alone (5000 J2/5 kg soil) than with the *S. sclerotiorum* alone (3 g mycelium/5 kg soil) (Table 11 and 12; Fig. 7; Plate 7). Highest suppression of test parameters was observed in plants simultaneously inoculated with both pathogens followed by plants inoculated with nematodes one week prior to fungus and fungus one week prior to nematodes, respectively.

All the treatments significantly ($P \leq 0.05$) reduced plant growth parameters as compared to uninoculated control. However, no differences ($P \leq 0.05$) were observed among all growth parameters between *M. incognita* prior to *S. sclerotiorum* inoculation and fungus prior to nematode inoculations and in shoot fresh weight between nematode alone and fungus alone. The maximum reduction in shoot height (32.6%), shoot dry weight (48.2%) and roots/suckers dry weight (51.5%) was observed in simultaneous nematode and fungus inoculation and minimum reductions of 9.8, 20.5 and 21.7%, respectively, was observed in plants inoculated with the fungus alone.

The reproduction and infectivity of *M. incognita* were suppressed in the presence of *S. sclerotiorum* while extent of roots/suckers infection by the fungus increased in the presence of nematode (Table 11; Fig. 8). Highest reproduction rate (19.71) of *M. incognita* and root-knot index (1.90) were observed in plants inoculated with the nematode alone, followed by nematode inoculation prior to fungus (16.42 and 1.65), nematode and fungus simultaneously (13.4 and 1.4) and fungus prior to nematode (11.9 and 1.25), respectively. The highest roots/suckers infection by fungus (62.0%) was observed in plants inoculated simultaneously with nematode and fungus followed by nematode prior to fungus (55.0%), fungus prior to nematode (48.0%) and fungus alone (42.5%), respectively.

Table 11: Effect of *Meloidogyne incognita* (5000 J2/5kg soil) and *Sclerotinia sclerotiorum* (3 g mycelium/5 kg soil) on nematode multiplication, root-knot disease development, percent roots and suckers infection by the fungus and growth of *Mentha arvensis* cv. Gomti.[a]

Treatments	Shoot height (cm)	Plant fresh weight (g)			Plant dry weight (g)			Final nematode population			Reproduction factor	[b]Root-knot index	[c]Roots & suckers infection
		Shoot	Roots & suckers	Total	Shoot	Roots & suckers	Total	Roots & suckers	Soil (5 kg)	Total			
Uninoculated control	77.2	142.3	134.1	276.4	34.0	25.8	59.8	-	-	-	-	-	-
Nematode alone	62.4 (19.2)[d]	106.5 (25.16)	97.0 (27.7)	203.5 (26.4)	25.2 (25.9)	18.5 (28.3)	43.7 (26.9)	46560	52000	98560	19.71	1.90	-
Fungus alone	69.6 (9.8)	114.1 (19.8)	106.9 (20.9)	220.9 (20.1)	27.0 (20.5)	20.2 (21.7)	47.2 (21.1)	-	-	-	-	-	42.50
Nematode + Fungus	52.0 (32.6)	74.5 (47.6)	65.6 (51.1)	140.1 (49.3)	17.6 (48.2)	12.5 (51.5)	30.1 (49.7)	28864	38000	66864	13.37	1.45	62.00
[e]Nematode (Pre) + Fungus (Post)	56.2 (27.2)	94.9 (33.3)	87.2 (35.0)	182.2 (34.1)	22.6 (33.5)	16.7 (35.3)	39.3 (34.3)	40112	42000	82112	16.42	1.65	55.00
[f]Fungus (Pre) + Nematode (Post)	58.5 (24.2)	100.0 (29.7)	92.0 (31.4)	192.0 (30.5)	23.8 (30.0)	17.5 (32.2)	41.3 (30.9)	29440 -	30000 -	59440 -	11.89 -	1.25. -	48.00 -
L.S.D. $_{0.05}$	2.6	10.7	4.1	12.8	1.0	0.8	1.4	196.1	836.7	258.0	0.05	0.07	1.74
L.S.D. $_{0.01}$	3.6	14.7	5.5	17.4	1.3	1.0	1.8	267.4	1141.0	351.8	0.07	0.09	2.37

[a]Each value is an average of five replicates.
[b]Root-knot index: 0 = 0%, 1 = 1-25%, 2 = 26-50%, 3 = 51-75%, 4 = 76-100%.
[c]Percent roots and suckers infection by *S. sclerotiorum*.
[d]Figures in parentheses are percent reduction over uninoculated control.
[e]*M. incognita* inoculated seven days prior to *S. sclerotiorum*.
[f]*S. sclerotiorum* inoculated seven days prior to *M. incognita*.

Table 12: Effect of *Meloidogyne incognita* (5000 J2/5 kg soil) and *Sclerotinia sclerotiorum* (3 g mycelium/5 kg soil) on oil yield and biochemical changes in plants of *Mentha arvensis* cv. Gomti.[a]

Treatments	Oil yield (ml/g fresh herb)	Chlorophyll content (mg/g fresh leaves)			Total phenol (mg /g fresh leaves)	Total sugar (mg/g fresh leaves)
		Chl a	Chl b	Total chl		
Uninoculated control	0.78	1.17	0.61	1.79	12.35	15.15
Nematode alone	0.61 (21.79)[b]	0.90 (23.08)	0.46 (24.59)	1.37 (23.50)	9.25 (25.10)	12.10 (20.13)
Fungus alone	0.72 (7.69)	1.08 (7.69)	0.55 (8.20)	1.64 (8.38)	11.00 (10.93)	13.75 (9.24)
Nematode + Fungus	0.52 (33.33)	0.75 (35.89)	0.38 (37.70)	1.14 (36.31)	7.75 (37.25)	10.15 (32.34)
[c]Nematode (Pre) + Fungus (Post)	0.56 (28.20)	0.80 (31.62)	0.41 (32.78)	1.22 (31.84)	8.15 (34.01)	10.75 (29.04)
[d]Fungus (Pre) + Nematode (Post)	0.58 (25.64)	0.86 (26.47)	0.44 (27.87)	1.30 (27.37)	8.78 (28.91)	11.50 (24.09)
$L.S.D._{0.05}$	0.017	0.03	0.02	0.019	0.05	0.04
$L.S.D._{0.01}$	0.023	0.05	0.03	0.026	0.06	0.06

[a]Each value is an average of five replicates.
[b]Figures in parentheses are percent reduction over uninoculated control.
[c]*M. incognita* inoculated seven days prior to *S. sclerotiorum.*
[d]*S. sclerotiorum* inoculated seven days prior to *M. incognita.*

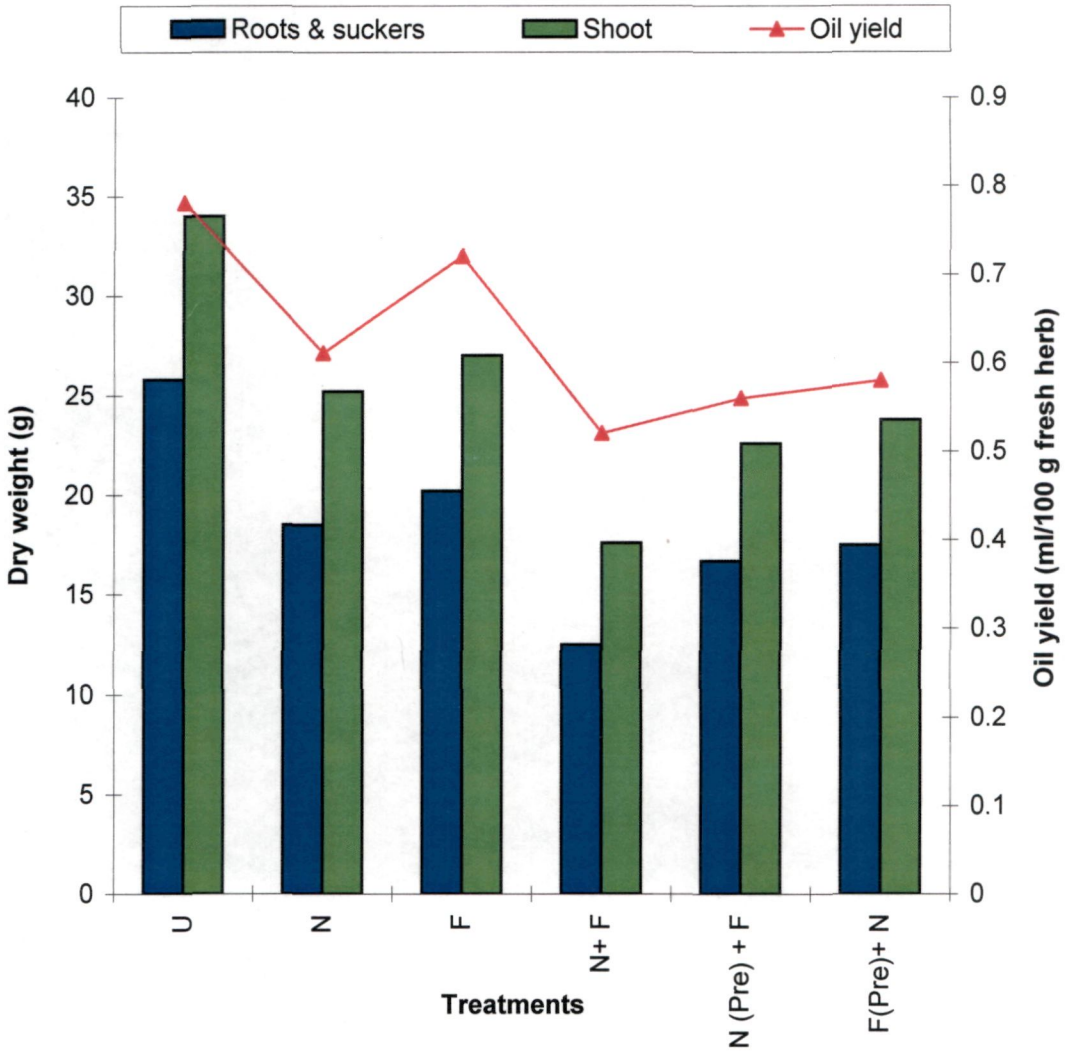


Fig. 7: Effect of *M. incognita* (5000 J2/5 kg soil) and *S. sclerotiorum* (3 g mycelium/5 kg soil) on the growth and oil yield of *M. arvensis* cv. Gomti.

U = Uninoculated
N = Nematode alone
F = Fungus alone
N + F = Nematode and Fungus inoculated simultaneously
N (Pre) + F = Nematode inoculated seven days prior to Fungus
F (Pre) + N = Fungus inoculated seven days prior to Nematode

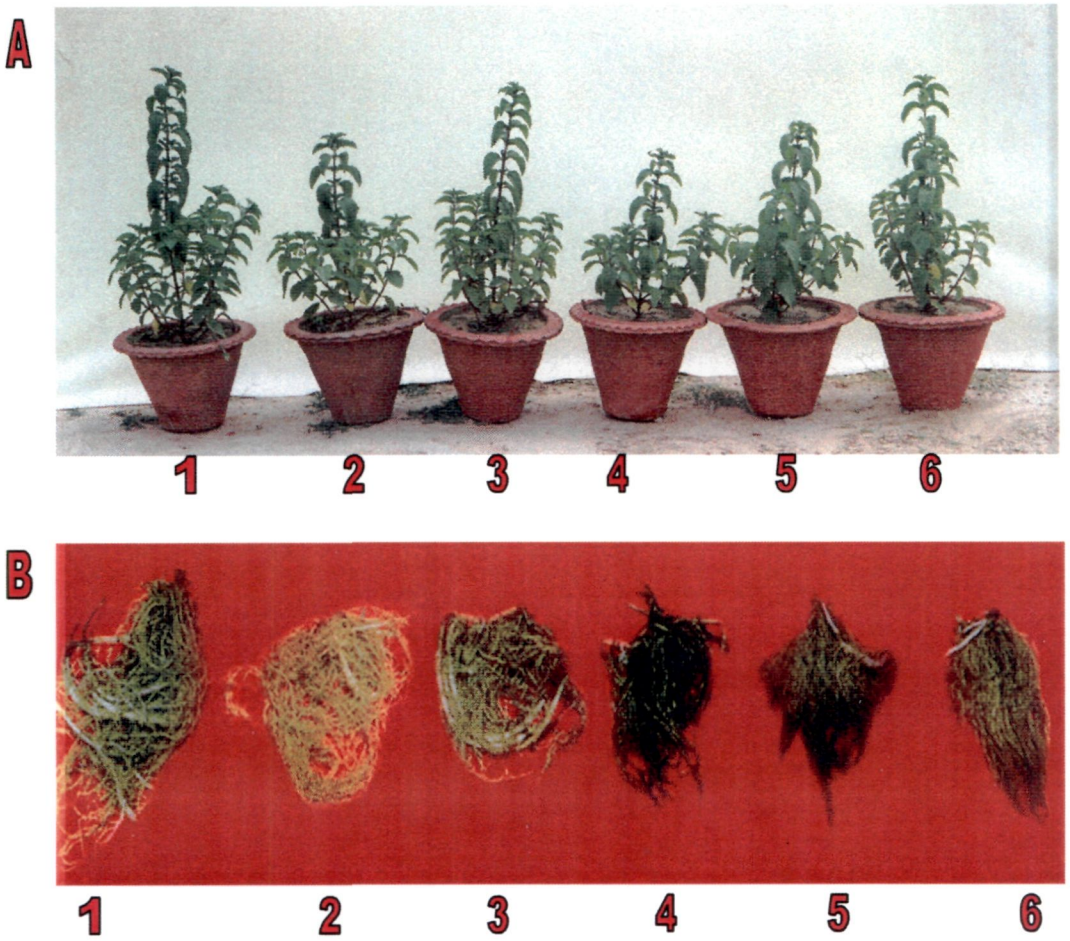


Plate 7: Effect of *M. incognita* and *S. sclerotiorum* alone or combined inoculation on aerial growth (A) and roots and suckers development (B) of *M. arvensis* cv. Gomti.

1 = Uninoculated control

2 = 5000 J2/pot

3 = 3 g mycelium/pot

4 = 5000 J2 + 3 g mycelium/pot

5 = 5000 J2 inoculated a week prior to 3 g mycelium/pot

6 = 5000 J2 inoculated a week after 3 g mycelium/pot

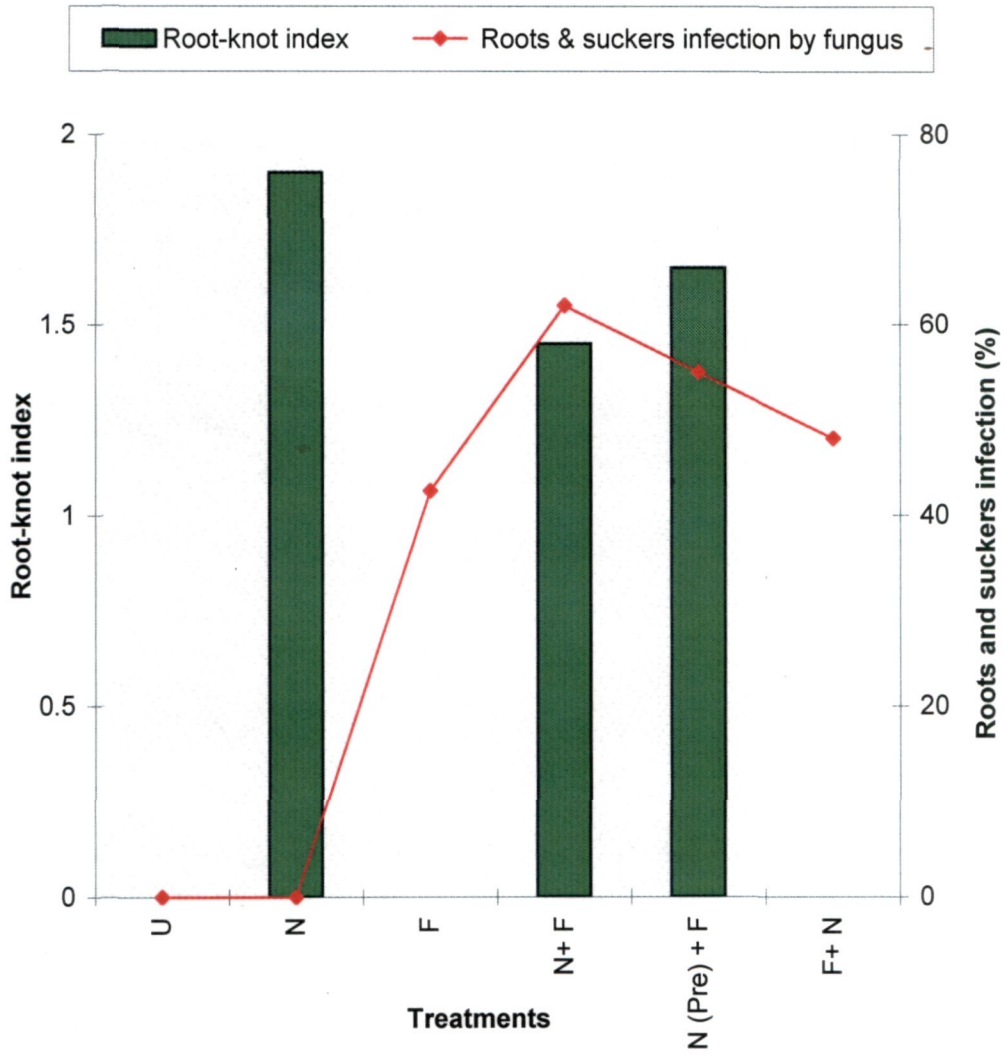


Fig. 8: Effect of *M. incognita* (5000 J2/5 kg soil) and *S. sclerotiorum* (3 g mycelium/5 kg soil) on disease development on *M. arvensis* cv. Gomti.

Root-knot index:- 0 = 0%, 1 = 1-25%, 2 = 26-50%, 3 = 51-75%, 4 = 76-100%

U = Uninoculated
N = Nematode alone
F = Fungus alone
N + F = Nematode and Fungus inoculated simultaneously
N (Pre) + F = Nematode inoculated seven days prior to Fungus
F (Pre) + N = Fungus inoculated seven days prior to Nematode

Analyses of the data indicated that the effect of all the treatments on above-mentioned parameters was highly significant ($P \leq 0.01$).

The reduction in oil yield, chlorophyll (a, b and total), total phenol and total sugar content of inoculated plants was significant as compared to uninoculated control (Table 12; Fig. 7). The maximum reduction in oil yield, chlorophyll a, chlorophyll b, total chlorophyll, total phenol and total sugar (33.3, 35.9, 37.7, 36.3, 37.2 and 32.3% respectively) was observed in plants inoculated with *M. incognita* and *S. sclerotiorum* simultaneously followed by the nematode prior to fungus inoculation, fungus prior to nematode, nematode alone and fungus alone respectively as compared to uninoculated control.

DISCUSSION

Scanning of literature shows that rarely any report is available on the interactive effect of *M. incognita* and *S. sclerotiorum* on crops. Several reports are available regarding the increased reduction in plant growth and yield in plants inoculated with more than one pathogen as compared to plants inoculated with a single pathogen. Especially, when root-knot nematode and fungus interact on a host, damage varies with the sequence of inoculations.

In the present study, the effect of sequential, simultaneous and single inoculation of *M. incognita* and *S. sclerotiorum* was tested on *M. arvensis* cv. Gomti. In general, the highest reduction in plant growth, and plant chemicals measured were found in plants inoculated with the nematode and fungus simultaneously followed by nematode prior to fungus and fungus prior to nematode inoculations, respectively. Highest reproduction factor and root-knot index were observed in plants inoculated with nematode alone and highest percent roots/suckers infection due to fungus was witnessed in nematode and fungus inoculated simultaneously.

The greatest reduction in plant growth parameters and yield by the simultaneous inoculation of nematode and fungus are also observed in various complexes involving *M. incognita* and *R. solani* on eggplant (Haseeb, 1983); *Sclerotium rolfsii* on betelvine (Sitaramaiah and Devi, 1990); *Thielaviopsis*

basicola on cotton (Walker *et al.,* 1999); *F. udum* on pigeonpea (Perveen *et al.*, 1999 a, b). However, several other workers (Porter and Powell, 1967; Khan and Muller, 1982; Singh and Goswami, 2001) also found the highest damage with the prior inoculation of nematode to that of fungus. But no report of highest damage is available when fungus was inoculated prior to nematode inoculations. This indicates that nematode prior or simultaneous inoculation enhanced the fungal activities on different susceptible as well as fungus tolerant hosts (Jenkins and Coursen, 1957; Perry, 1961; Webster, 1985; Kassab and Ali, 1995).

The reproduction and infectivity of *M. incognita* were observed to be surpressed in the presence of fungus while extent of root infection by fungus was increased in the presence of nematodes. Similarly, reduction in the population of *M. incognita* has also been reported in concomitant infection with *Pythium aphanidermatum* on lettuce by Johnson and Littrell (1970); with *R. solani* on potato by Sharma and Gill (1979) and with *R. solani* on soybean by Anwar and Khan (2002). The possible explanations for the reduction in the population of *M. incognita* in presence of *S. sclerotiorum* might be due to the damage of root tissues by the fungus before the completion of life cycle of nematodes it could be due to the fact that *S. sclerotiorum* produces pectolytic enzymes, which macerate tissues and directly or indirectly, damage the cell membranes leading to the death of cells (Bateman and Basham, 1976), another possible reason for the reduction in nematode population in the presence of fungus might be the antagonist inhibitory effect of the fungus on subsequent hatching of egg masses (Golden and Van Gundy, 1975; Sharma and Gill, 1979). In present studies reduction in *M. incognita* population was probably more due to the antagonistic effect of the fungus than that due to damage to root tissues.

The increased root infection by the fungus in presence of nematodes suggests a predisposition of *M. arvensis* cv. Gomti roots by *M. incognita* for subsequent damage by *S. sclerotiorum.* Golden and Van Gundy (1975) expressed similar views while experimenting with *M. incognita* and *R. solani* on okra and tomato. They hypothesized that the leakage of nutrient from the root was responsible for attracting the fungus to the nematode induced galls. In

addition, nematode might have provided infection courts through which the fungus entry might have been facilitated as suggested by Smith (1954) in case of Fusarium wilt and root-knot nematode complex on cotton.

The maximum reduction in oil yield, chlorophyll (a, b and total), total phenol and total sugar was observed in simultaneous inoculation of nematode and fungus followed by nematode prior to fungus, fungus prior to nematode, nematode alone and fungus alone, respectively. Anwar and Khan (2002) reported severe decreases in concentration of chlorophyll (a, b and total), oil and protein contents with simultaneous inoculation of *M. incognita* and *R. solani* on soybean followed by nematode prior and after the inoculation of fungus, nematode alone and fungus alone, respectively. Similar results were also reported by Jain and Goswami (2002) on *M. incognita* and *F. oxysporum* f. sp. *lycopersici* disease complex of tomato.

The result of the present investigation suggests that *M. incognita* and *S. sclerotiorum* together cause greater damage to *M. arvensis* cv. Gomti than either of them alone and in order to obtain the maximum yield the prophylactic management procedures must be applied against these pathogens.

Chapter 5

Effect of Abiotic Factors

6. Effect of different pH levels on disease development, nematode multiplication, growth, oil yield and biochemical changes in *M. incognita* and/or *S. sclerotiorum* inoculated plants of *M. arvensis* cv. Gomti

The changes in pH are closely related to the changes in other factors and it is difficult to separate the influence of one from the other. The pH of soil has been considered to be an important factor on the occurrence and severity of plant diseases caused by certain soil-borne pathogens.

REVIEW OF LITERATURE

Plant-parasitic nematodes

Although much is known about pH yet less is known about its effect on nematodes. Since pH is important biologically, it seems logical to expect that nematodes be affected at diverse ranges of pH changes, as are other forms of life. Although nematodes seem to tolerate wide pH ranges, there are limits in which the hydrogen or hydroxyl ions, or other ions affected by pH, become toxic (Norton, 1978).

The extent of damage caused by plant-parasitic nematodes to different crops have been found to be dependent upon the susceptibility of host, age and vigour of the host at the time of infection, species and densities of nematodes, temperature, moisture, oxygen, soil fertility, physical and chemical properties of soil (Wallace, 1969; Webster, 1972; Norton, 1978; Melakeberhan *et al.*, 1984, 1985; Haseeb *et al.*, 1990; Haseeb and Shukla, 1994, 1995, 1996, 2000b, 2001a). Among these, pH is considered to be an important factor, which directly and indirectly influences the plant growth and plant nematode relationship to a great extent (Norton, 1978; Kimpinski and Willis, 1981; Shukla *et al.*, 1997a, b, 1998; Haseeb *et al.*, 1999b).

Characteristics of the environment in which plant pathogenic nematodes occur, affect population densities, distribution and activities of these obligate parasites. In general, soil chemical factors such as pH and mineral nutrition

concentrations that are optimum for these parasites are very similar to those that optimize growth of the host (Norton, 1978, 1979).

Banage and Visser (1965) studied the effect of some fatty acid solutions (formic, acetic, propionic, butyric and valaric) between 1-10 pH on *Dorylaimus* sp. and found that formic acid was less toxic at any concentration, whereas all other acids were similar to each other in their effect.

An early study of Wallace (1966) indicated that *Meloidogyne* spp. survive, hatch and reproduce between 4.0-8.0 soil pH. However, the eggs of *M. javanica* hatch maximum between 6.4 to 7.0 soil pH and inhibit below 5.2 pH.

Later on, Morgan and MacLean (1968) studied the influence of soil pH on *P. penetrans* infecting vetch seedlings and found the most suitable pH range from 5.5 to 5.8 for the multiplication of *P. penetrans.* The multiplication of the nematode was maintained between the soil pH of 4.8 to 6.6, but at further increase in pH, the multiplication of nematode was significantly decreased. Apparently the root system of vetch was not affected by soil pH but it had some inhibitory effect on crop growth between pH 7.1-7.5.

Brzeski and Dowe (1969), while surveying the cabbage fields in Poland infested with *Tylenchorhynchus dubius* and having different ranges of soil pH, noted more frequency of occurrence of *T. dubius* in acidic soil. They also studied the effect of pH (3.0-8.0) on the multiplication of *T. dubius* and noted that the decrease in soil pH, exhibited an increase in nematode multiplication. However, the highest hatching of the eggs was observed at pH 7.0 and it was inhibited at very low pH 3.0.

Burns (1971) made an attempt to study the effect of three different pH levels (4.0, 6.0 and 8.0) on *P. alleni* population infesting soybean and reported that the highest colonization of the nematode in roots was found at pH 6.0.

Willis (1972) investigated the effect of different ranges of soil pH (4.4, 5.2, 6.4 and 7.3) in fine sandy loam on the multiplication of *P. penetrans* and yield of alfalfa cv. Vernal and reported that the nematode reproduction was found to be significantly greater at soil pH of 5.2 and 6.4. He further noticed a

negative correlation on the growth of alfalfa root however; the yield of alfalfa was significantly reduced at pH 5.2 and 6.4.

Azmi and Jairajpuri (1976), while studying the influence of pH on activities of *Hemicriconemoides mangiferae,* indicated the influence of pH on the activity and orientation of the nematode. The higher concentrations witnessed a toxic effect and a gradual decrease in the level of pH brought out in enhanced activity of the nematodes. Whereas, the pH range between 5.8 to 6.4 was not found suitable for the activity of *H. mangiferae*

Kimpinski and Willis (1981) studied the effect of different pH levels (5.0, 6.0 and 7.0) on the movement, reproduction and survival of *P. penetrans* and *P. crenatus* in alfalfa and timothy, and noted a rapid decrease in the population of *P. crenatus* at higher soil pH, whereas, *P. penetrans* showed a significant increase in the population at the same pH level. Nematode reproduction was decreased with the increase in pH level up to 6.9. Yields were significantly lower in alfalfa infected with *P. penetrans* than in plants harbouring *P. crenatus* at all the pH levels. Timothy yields did not differ in their response to both nematode species and yields of both host plants increased with the increase in soil pH.

Malik and Jairajpuri (1984) stated that the survival period of the *Xiphinema americanum* was highest at pH 6.0-6.6 both in buffer solution and tap water. However, the pH range from 5.4 to 7.4 was found to be the most suitable for the nematode activity. Whereas, pH range between 2 to 3 of phosphate buffer proved to be highly toxic to adults and juveniles.

Hakim *et al.* (1985) studied the effect of different pH levels (3.0, 7.0 and 9.0) on root-knot development and morphometric of *M. incognita* on tomato cv. Marglobe. They found that the nematode reproduction was found to be highest at pH 7.0 followed by pH 9.0 and 3.0 respectively. The measurements like body width and length, neck length, width and length of median bulb were significantly higher at soil pH 7.0 than those obtained from plants grown at either pH levels.

Gnanapragasam (1987) determined the effect of soil acidity on the population levels of *Pratylenchus loosi* and yield of tea from over 600 different fields and locations in Sri Lanka. There was no correlation between soil pH and population of nematode in soil. However, at lower pH, even at low population of *P. loosi* caused significant damage than those in soils with an optimum pH.

Volcy (1990) conducted a pot experiment to study the effect of different pH levels (4.1, 4.8, 6.0 and 7.3) on the development of root- knot caused by *M. incognita* in tomato grown in clay loam soil. The pH levels had no effect on the duration of life cycle of the nematode but egg laying was influenced by pH. The fecundity of the nematode was affected with the production of eggs tending to increase with the increase in pH, whereas, these activities suppressed at pH 4.1.

Sarah *et al.* (1991) studied the effect of different pH levels (3.8-6.2) on the population build up of *P. brachyurus* in pineapple roots at Ivory Coast. The nematode populations were developed most rapidly in pineapple roots in plots, which had lowest soil pH. The nematode population decreased gradually during the growth of the crop and disappeared after flower induction.

Khan and Khan (1992) determined the effect of artificially created soil salinity levels by additions of NaCl and $NaHCO_3$ in soil on penetration and development of *M. javanica* in the roots of cucumber and okra. Penetration of second-stage juveniles in cucumber and okra roots and development of juveniles into adult females were impaired by the salinity levels. A direct correlation existed between concentration of the salts and number of root-ingressed juveniles. Production of egg masses was delayed and their number was significantly reduced. The soil salinity impeded nematode growth and reproduction, which in turn reduced the harmful effects of the nematode on plants.

Haverkort *et al.* (1993) investigated the effect of different soil pH levels (4.5-6.5) on multiplication of *Globodera pallida* and yield of potato under field conditions and in a semi controlled environment. In field experiment, potato yield decreased from 45 to 33 t/ha at pH 4.5 and pH 6.5 respectively. The nematode population was however, decreased from about 18 to 9 juveniles/g

soil. In container experiment, tuber yield was about 11% less at pH 6.5 than at pH 4.5 in the absence of nematodes, but about 44% less yield was recorded at an initial population of 27 juveniles/g soil. The increase in soil pH from 4.5 to 6.5 caused reduction in the yield of potato in control plots as well as in pots, but not so for the nematode population.

Choudhury and Phookan (1995) reported that the populations of *Cephalenchus leptus, H. dihystera, Macroposthonia medani, M. incognita* and *T. leviterminalis* were observed to be maximum at pH levels of 5.0-5.9. Whereas, *Hop. indicus* population was maximum at pH above 6.9 and *Pratylenchus* sp. at 4.0-4.9 pH.

Khan *et al.* (1997) studied the effect of soil salinity in presence of NaCl and $NaHCO_3$ and *M. incognita* on the growth of okra and cucumber, root galls and fecundity of nematode. They observed that the soil salinity and *M. incognita* reduced the plant growth. It has also been noticed that there was a corresponding decrease in number of galls, number of eggs/egg masses with the increase in concentration of the salts.

Shukla *et al.* (1997a, b; 1998), while determining the effect of pH levels on the reproduction of *P. thornei* and yield of *M. spicata, M. citrata* and *M. piperita,* reported that the reduction in the growth of mint plants and reproduction of *P. thornei* was highest at pH 6.0 followed by pH 9.0 and pH 3.0 respectively.

Urek (1998) studied the influence of soil pH on nematode population in soil. He reported that an acidic soil was found to be more suitable for *P. crenatus* and for other plant-parasitic nematodes, whereas, a less acidic or neutral soil was preferred by bacteriovorous free-living nematodes.

Singh *et al.* (1998) studied the effect of different pH levels on the development and morphometrics of *Heterodera zeae* on maize in pot conditions. They observed that the plant growth characters were at its maximum at pH 9.0, while maximum nematode population was recorded at neutral pH level (7.0). However, the morphometrics characters of the nematode were significantly decreased at pH 9.0 and 5.0 as compared to neutral pH (7.0).

Haseeb *et al.* (1999a) made an attempt to study the effect of different pH levels (3.0, 4.5, 6.0, 7.5, 9.0 and 10.5) and plant-parasitic nematodes on the survival and growth of *H. niger*. They observed that the transplanted seedlings of *H. niger* survived at all pH levels irrespective of the presence or absence of the plant-parasitic nematodes. The growth of control plants was found in inversely proportionate to pH levels, while presence of nematodes altered the trend of growth. The greatest nematode reproduction and reduction in plant length, fresh and dry weight was observed at pH 6.0 followed by pH 7.5, 9.0, 4.5, 10.5 and 3.0 respectively. *M. incognita, H. indicus* and *T. vulgaris* reproduced well, while *P. thornei* and *Tylenchus* sp. suppressed in the rhizosphere of *H. niger.*

Haseeb and Shukla (2000a) studied the relationships between different pH levels, reproduction, damage potential of *M. incognita,* growth and oil yield of *M. arvensis* cv. Hy-77. They noticed a direct proportional relationship between the pH levels and severity of the disease. Reproduction of *M. incognita* was highest at pH 6.0 followed by 9.0 and 3.0 respectively.

Sharma (2001) conducted an experiment to study the infestation potential of *M. incognita* on French bean grown in different types of soil with different pH. They observed that the neutral soil (pH 7.09) supported the least infestation of the nematode as compared to alkaline (pH 7.97-8.69) or acidic soils (pH 6.15). The multiplication of the nematode was lowest in the soil with highest organic content.

Prasad *et al.* (2001) studied the effect of different soil pH on the development and morphometrics of *H. avenae* and reported that the maximum nematode population was observed at neutral pH level (7.0). The morphometric characters decreased at pH levels 9.0 and 5.0 as compared to neutral pH.

Phytopathogenic fungi

The pH of soil has been considered to be an important factor on the occurrence and severity of plant diseases caused by certain soil-borne pathogens. In many diseases, this effect seems to be principally on the pathogen, however, in some diseases, a weakening of the host through altered nutrition, is induced

by the soil acidity which may influence the incidence and severity of the disease (Agrios, 2000).

Tanrikut and Vaughan (1951) determined the effect of hydrogen-ion concentration on the rate and growth character of *S. sclerotiorum* in culture and reported that all isolates made some growth at pH 2.0, whereas, at 2.4 pH a fairly more growth rate of all the isolates was observed. However, the rapid growth of the fungus was observed at pH 3.0 to 8.9. The rate of growth of all culture was retarded at pH 9.6 but very slight growth of it occurred at pH 10.2. Interestingly, there was no growth at pH 10.5 or above.

Irvine and Valleau (1954) stated that the tobacco crop growing in the fields having soil pH of 6.5 or above was found to be more susceptible to black shank disease caused by *Phytophthora parasitica* var. *nicotianae* than the crop raised in fields with soil pH of 5.6 or below.

Echandi and Walker (1957) studied the relationship of different pH levels (3.0, 4.0, 5.0, 6.0 and 7.0) to pectolytic enzymes produced by *S. sclerotiorum* and concluded that the optimum pH for enzyme activity seems to be at pH 4.0

Troutman and LaPrade (1962) reported that the incidence of black shank disease of tobacco due to *P. parasitica* var. *nicotianae* was much higher in soil at pH 7.0 than that at pH 4.0. In another study, Mathur (1962) found that the maximum disease intensity of stem gall of coriander due to *P. macrosporus* was at pH 7.5.

Mathur and Sinha (1966) studied the disease development in guar and gram in presence of *S. rolfsii* under different pH conditions and recorded maximum expression of disease at acidic pH (6.0). An increase in the pH level, however, decreased the severity of disease.

Lumsden (1969) studied the activity of cellulase produced by *S. sclerotiorum* and concluded that the cellulase extracted from the fungus cultured on autoclaved bean hypocotyl medium and from diseased tissue of *P. vulgaris* showed a narrow pH range for the enzyme activity with an optimum pH of 3.0.

The activity of cellulase was greatly reduced at pH 5.0 and above, whereas, the cellulase extracted from the healthy tissues exhibited its activity at pH 5.0.

Shirata *et al.* (1980) reported that the optimum pH for the activity of cell wall degrading enzymes produced by *S. sclerotiorum* was found to be at 3.0-3.5. The mulberry shoot inoculated with culture filtrate of *S. sclerotiorum* showed the formation of brown lesion at pH 4.5 but not at pH 8.0.

Khare *et al.* (1981) reported that the growth and sclerotia formation by *S. minor* were observed to be excellent on PDA, carrot agar and glucose-peptone agar media and the pH range for the growth and scletrotia formation was found to be between 2.5 to 8.0, with optimum at pH 4.0. Lukas and Gilly (1982) reported that the optimum pH for mycelial growth of *S. sclerotiorum* was 4.4 while for sclerotial development was observed to be the optimum at pH 3.0.

Sharma *et al.* (1984) studied the protease activity of *S. sclerotiorum* causing stalk rot of cauliflower in Czapek's medium. They reported that the maximum production of protease was found at pH 5.0 followed by pH 6.0. However, there was no significant difference in mycelial growth at these pH levels.

Litkei and Voros (1984) reported that at pH 2.0 – 4.0, 80-84% sclerotia of *S. sclerotiorum* germinated, however, at this pH only mycelial growth occurred. Whereas, at pH 8.0, germination of sclerotia was 84% and only apothecia were formed. However, at pH 5.0 – 6.0 the sclerotial germination was 36-40% and at this pH sclerotia were germinated to apothecia as well as to mycelium.

Iliescu *et al.* (1988) observed that the *S. sclerotiorum* and *S. minor* isolated from sunflower, grew better at temperature ranged from 20-24 °C and the production of sclerotia of both the fungus was found maximum at pH 6.5.

Singh and Gandhi (1991) reported that *S. rolfsii* caused maximum seedling mortality (94%) in guar at pH 6.1 and with the increase in pH the disease severity was decreased significantly. However, mortality of the seedlings was found to be significantly less in alkaline range of soil pH i.e. 7.5 and 8.4.

Kumar and Mishra (1993) reported that the pH range for spore germination of *Drechslera oryzae* was found between pH 5.0 to 8.0. However, the optimum pH for spore germination was between the pH of 6.8 to 7.0.

Lee *et al.* (1995) reported that *B.* (*Sclerotinia*) *squamosa* grown well at pH 4.0-8.0 on PDA medium and pH 5.0 was observed optimum for mycelial growth. Shi *et al.* (1999) studied the effect of pH on *S. sclerotiorum* germination and mycelial growth on PDA medium. They observed that the best pH for both was 4.7-8.5, whereas, sclerotial formation was found higher at pH 4.7-10.5.

Maheshwari *et al.* (2000) studied the effect of temperature and pH on growth and sporulation of *A. alternata* an incitant of leaf spot of Dolichos bean and reported optimum temperature 28 °C and pH 6.5 for the growth of the fungus. The minimum growth of the fungus was recorded at the temperature of 5 °C and pH 10.5; however, its sporulation was highest at 25 - 30°C and pH levels of 5.5 to 6.5.

Ahmad *et al.* (2002) determined the effect of different pH levels viz. 4.0, 5.0, 6.0 and 7.0 on the growth, sporulation, biomass and gibberellic acid (GA3) production of *Gibberella fujikuroi*-52. They reported that the maximum growth, sporulation and biomass of the fungus were observed at pH 5.0. The most suitable range for the maximum production of GA3 was between pH 4.0-5.0.

Tripathi *et al.* (2002) studied the response of *P. macrosporus* and yield of coriander to different soil pH levels (5.5, 6.5, 7.5, 8.5, 9.5 and 10.0). They reported that the highest disease intensity was found at soil pH of 7.5 and lowest at pH 5.5, but at this pH, the growth characters and yield of plants was adversely affected.

MATERIALS AND METHODS

6.1 Preparation of pots and sand

The inner surface of 30-cm-diameter clay pots was painted with black enamel paint. It was done so to avoid the leaching of minerals from the pots.

Dry sand was sieved through 710-μm openings sieve to remove coarse particles, stones and gravels. The sand was treated with 20% hydrochloric acid (500 ml/kg sand) for 24 h in plastic containers. After that containers were left for further 24 h in the running tap water and thereafter the sand was washed for 10-12 times by stirring thoroughly in tap water. The washed sand was dried on a cleaned concrete platform. The painted clay pots were filled with the dry sand.

6.2 Preparation of Hoagland's solution and maintenance of pH

Complete Hoagland's solution was prepared by the procedure described by Hoagland and Arnon (1950). The composition of the nutrient solution (mg/l) was 102 K, 100 Ca, 70 N-NO_3, 16 S, 12 Mg, 9 Cl, 5 P, 0.52 B, 0.03 Mo, 0.10 Zn, 0.02 Cu, 0.05 Mn and Fe was supplied as Fe-EDTA (Ferric ethylene diamine tetracetate), at 5.6 μg/ml. Four pH levels (4.5, 6.0, 7.5 and 9.0) of Hoagland's solution were prepared by adding 1M NaOH or 20% HCl.

6.3 Transplanting, inoculation and recording of data

Transplanting, inoculation and recording of data was done in the same manner as described earlier in 2 and 4.

For each pH level, inoculation of *M. incognita* and *S. sclerotiorum* was done according to the following scheme:

(i) Uninoculated
(ii) 5000 J2
(iii) 3 g fungal mycelium
(iv) 5000 J2 + 3 g fungal mycelium simultaneously

Pots were irrigated every day with 100-300 ml full strength Hoagland's solution at specified pH levels. There were four replicates for each treatment. The experiment was laidout as a completely randomized block design.

RESULTS

Data presented in Table 13 indicates that soil pH had a significant effect on the growth and oil yield of *M. arvensis* cv. Gomti, both in the absence as well as in the presence of pathogens. An inversely proportional relationship was

Table 13: Effect of different pH levels on the growth and oil yield of uninoculated, *Meloidogyne incognita* (5000 J2/5 kg soil) and/or *Sclerotinia sclerotiorum* (3 g mycelium/5 kg soil) inoculated plants of *Mentha arvensis* cv. Gomti.[a]

Parameters and treatments	Soil pH				Mean	L.S.D.$_{0.05}$	
	4.5	6.0	7.5	9.0		Pathogen/ pH	Interaction
Shoot height (cm)							
Uninoculated control	77.1	71.5	66.5	59.1	68.5	1.05	2.11
Nematode alone	69.5 (9.8)[b]	60.0 (16.0)	53.2 (20.0)	43.5 (26.4)	56.5		
Fungus alone	61.1 (20.1)	59.0 (17.5)	59.5 (10.5)	54.0 (8.6)	58.4		
Nematode + Fungus	53.8 (30.2)	47.0 (34.3)	41.8 (37.1)	36.0 (39.0)	44.6		
Mean	65.4	59.4	55.2	48.1			
Shoot fresh weight (g)							
Uninoculated control	141.5	129.3	118.5	105.9	123.8	2.25	4.50
Nematode alone	128.4 (9.2)	104.0 (19.6)	89.0 (24.9)	74.0 (30.1)	98.9		
Fungus alone	105.5 (25.4)	102.0 (21.1)	95.8 (19.2)	90.0 (15.0)	98.3		
Nematode + Fungus	91.6 (35.3)	74.0 (42.8)	63.8 (46.2)	53.5 (49.5)	70.7		
Mean	116.7	102.3	91.8	80.8			
Roots and suckers fresh weight (g)							
Uninoculated control	138.0	120.5	115.5	102.0	119.0	2.23	4.46
Nematode alone	122.5 (11.2)	95.7 (20.6)	84.5 (26.8)	69.0 (32.3)	92.9		
Fungus alone	102.0 (26.1)	93.5 (22.4)	92.0 (20.3)	88.5 (13.2)	94.0		
Nematode + Fungus	85.0 (38.4)	68.5 (43.1)	59.5 (48.6)	50.0 (50.9)	65.7		
Mean	111.9	94.5	87.9	77.4			
Shoot dry weight (g)							
Uninoculated control	33.7	31.0	28.5	25.6	29.7	0.6	1.2
Nematode alone	30.2 (10.4)	24.7 (20.3)	21.5 (24.6)	17.8 (30.5)	23.5		
Fungus alone	25.0 (25.8)	24.0 (22.6)	22.7 (20.3)	21.6 (15.6)	23.3		
Nematode + Fungus	21.5 (36.2)	17.5 (43.5)	14.8 (48.1)	12.8 (50.0)	16.6		
Mean	27.6	24.3	21.9	19.4			
Roots and suckers dry weight (g)							
Uninoculated control	26.5	23.4	22.5	19.6	23.0	0.3	0.5
Nematode alone	23.3 (12.1)	18.5 (20.9)	16.3 (27.5)	15.2 (32.6)	18.3		
Fungus alone	19.3 (27.2)	18.0 (23.1)	17.7 (21.3)	16.9 (13.8)	18.0		
Nematode + Fungus	16.0 (39.6)	13.2 (45.6)	11.5 (48.9)	9.6 (51.0)	12.6		
Mean	21.3	18.3	17.0	15.3			
Oil yield (ml/100 g fresh herb)							
Uninoculated control	0.64	0.68	0.70	0.76	0.70	0.01	0.02
Nematode alone	0.62 (3.12)	0.58 (14.70)	0.56 (20.00)	0.56 (26.31)	0.57		
Fungus alone	0.58 (9.37)	0.62 (8.82)	0.65 (7.14)	0.72 (5.26)	0.64		
Nematode + Fungus	0.55 (14.06)	0.50 (26.47)	0.49 (30.00)	0.50 (34.21)	0.51		
Mean	0.60	0.59	0.59	0.63			

[a]Each value is an average of four replicates.

[b]Figures in parentheses are percent reduction over uninoculated control.

observed between pH levels and plant growth of uninoculated as well as inoculated plants. Relationship between pH levels and oil yield was directly proportional in uninoculated plants and plants inoculated with fungus alone, and inversely proportional in the presence of nematode.

Plants inoculated with *M. incognita* alone and *M. incognita* and *S. sclerotiorum* simultaneously, suffered maximum reduction of 26.4 and 39.0% in shoot height, 30.5 and 50.0% in shoot dry weight, 32.6 and 51.0% in root/sucker dry weight, 26.3 and 34.2% in oil yield respectively at pH 9.0. Whereas, plants inoculated with the fungus alone had maximum reduction of 21.1, 25.8, 27.2, and 9.37% for corresponding plant growth parameters at pH 4.5 (Fig. 9 and 10; Plate 8-11).

Analyses of data indicated that non-significant ($P \leq 0.05$) differences were observed between pH 6.0 and 7.5 in shoot height, fresh and dry weights of root/sucker, and between pH 4.5 and 6.0 in shoot fresh and dry weights of the plants inoculated with fungus alone. No difference in oil yield was observed between pH 7.5 and 9.0 in the plants inoculated with nematode alone and nematode and fungus simultaneously, and between pH 6.0 and 7.5 in plants inoculated with fungus alone. The reduction in growth of inoculated plants was significant ($P \leq 0.05$) as compared to uninoculated plants, irrespective of pH levels. However, shoot fresh weight showed difference between the plants inoculated with nematode alone and fungus alone at pH 6.0.

Nematode multiplication and disease development indicated that all pH levels had a significant ($P \leq 0.05$) effect on the reproduction of nematode and disease development by fungus. Highest final population of *M. incognita* in soil and roots/suckers, reproduction rate and root-knot index were observed at pH 9.0 followed by 7.5, 6.0 and 4.5 (Table 14; Fig.11), whereas, a reverse trend was found for percent roots/suckers infection by the *S. sclerotiorum*. At all pH levels, the populations of nematodes in sand and roots/suckers, reproduction rate and root-knot index were observed highest in plants inoculated with nematode alone, whereas, percent roots/suckers infection by the fungus was highest in the plants inoculated with fungus and nematode simultaneously. The highest reproduction

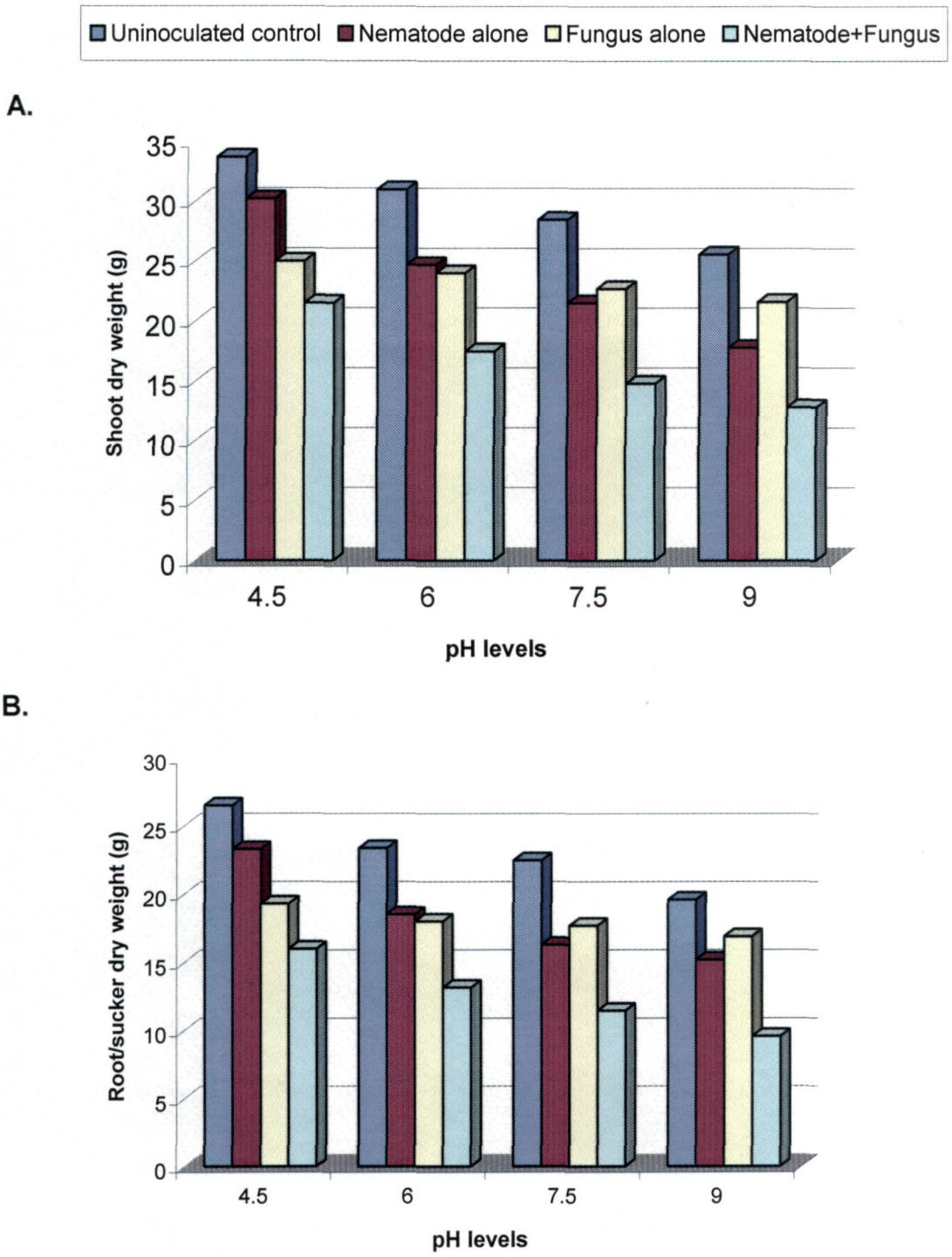


Fig. 9: Effect of different pH levels on shoot (A) and root/sucker (B) dry weights of uninoculated and inoculated (5000 J2 of *M. incognita* and/or 3 g mycelium of *S. sclerotiorum* /5 kg soil) plants of *M. arvensis* cv. Gomti.

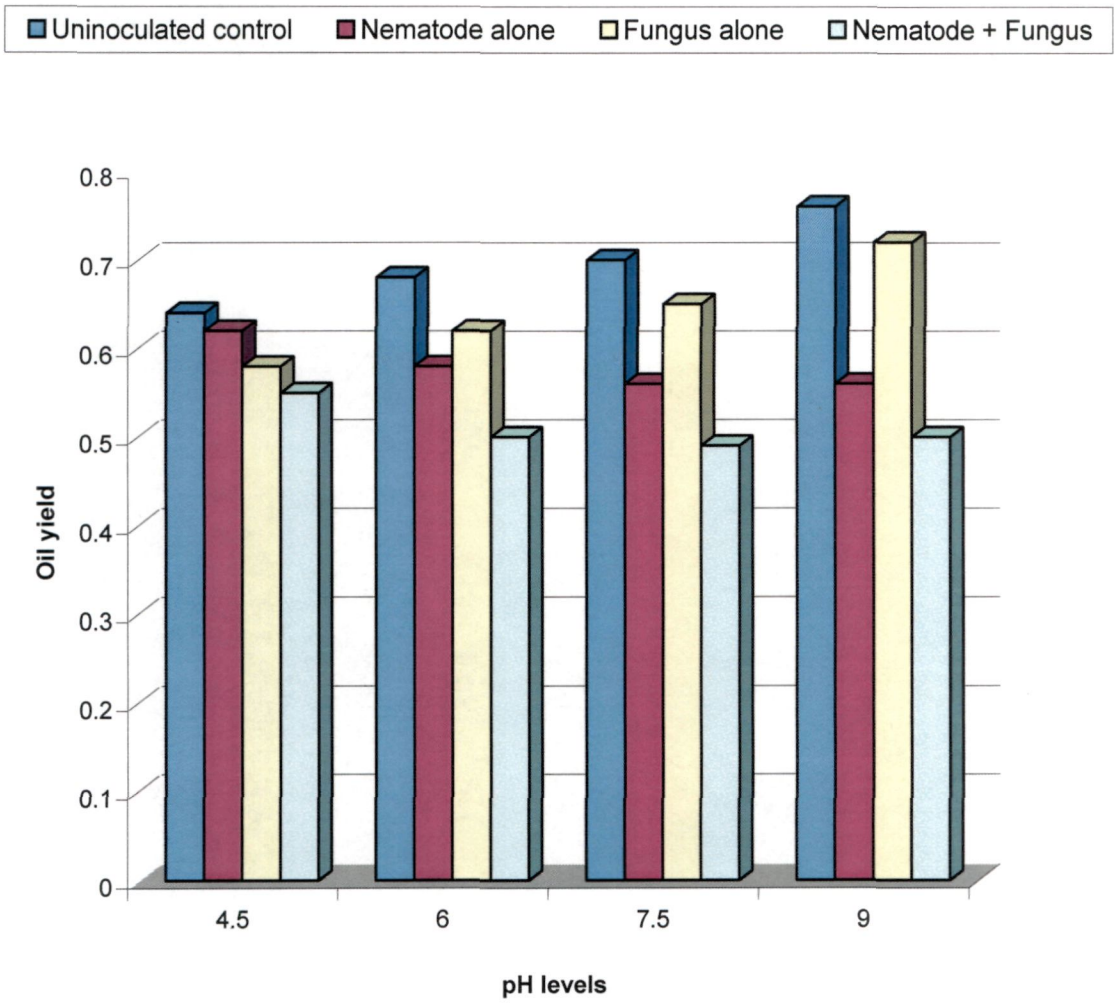


Fig. 10: Effect of different pH levels on oil yield of uninoculated and inoculated (5000 J2 of *M. incognita* and/or 3 g mycelium of *S. sclerotiorum* /5 kg soil) plants of *M. arvensis* cv. Gomti.

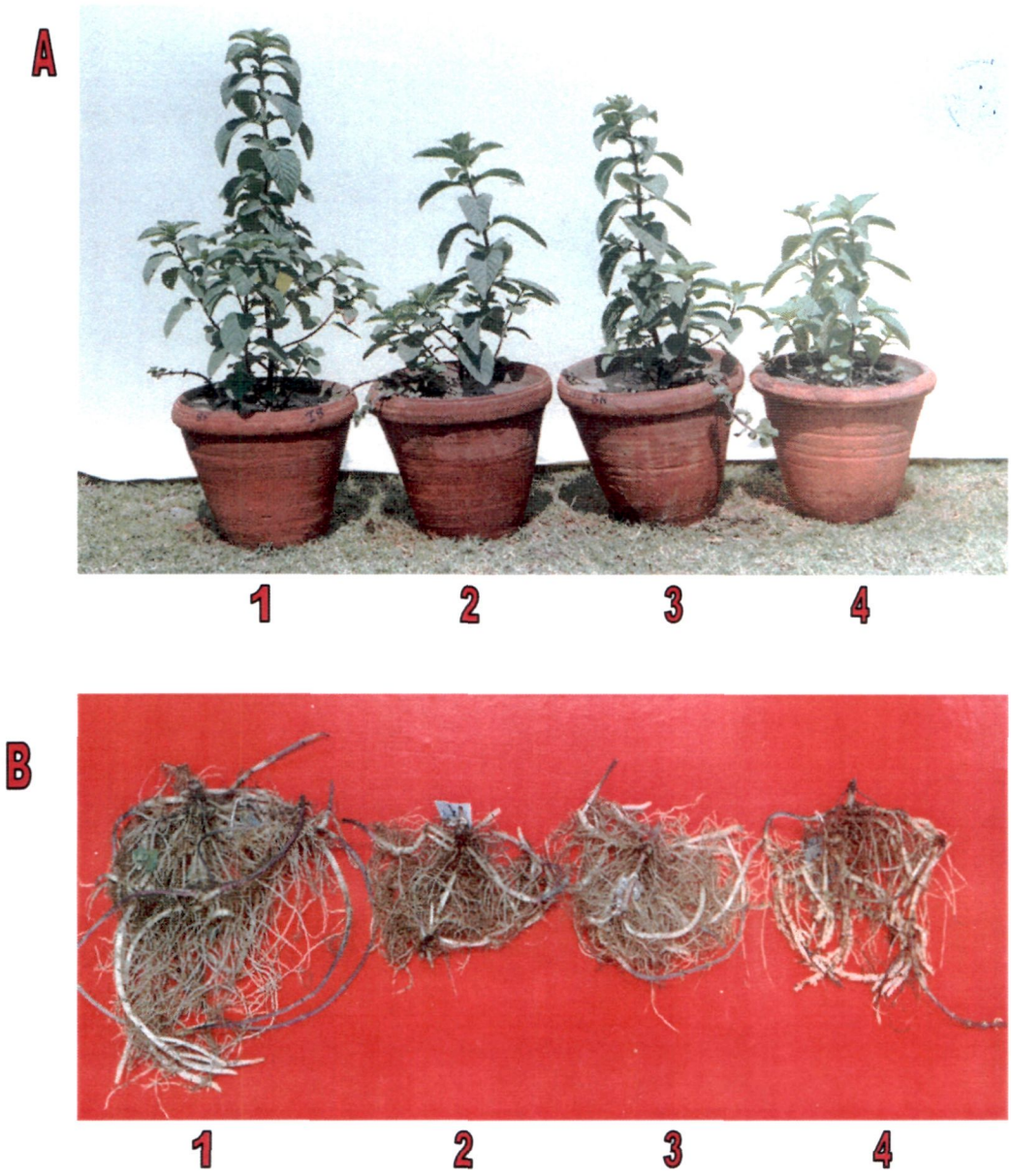


Plate 8: Effect of soil pH (4.5) on aerial growth (A) and roots and suckers development (B) of *M. arvensis* cv. Gomti.

1 = Uninoculated
2 = Inoculated with 3 g mycelium of *S. sclerotiorum*/pot
3 = Inoculated with 5000 J2 of *M. incognita*/pot
4 = Inoculated with 5000 J2 of *M. incognita* + 3 g mycelium of *S. sclerotiorum*/pot

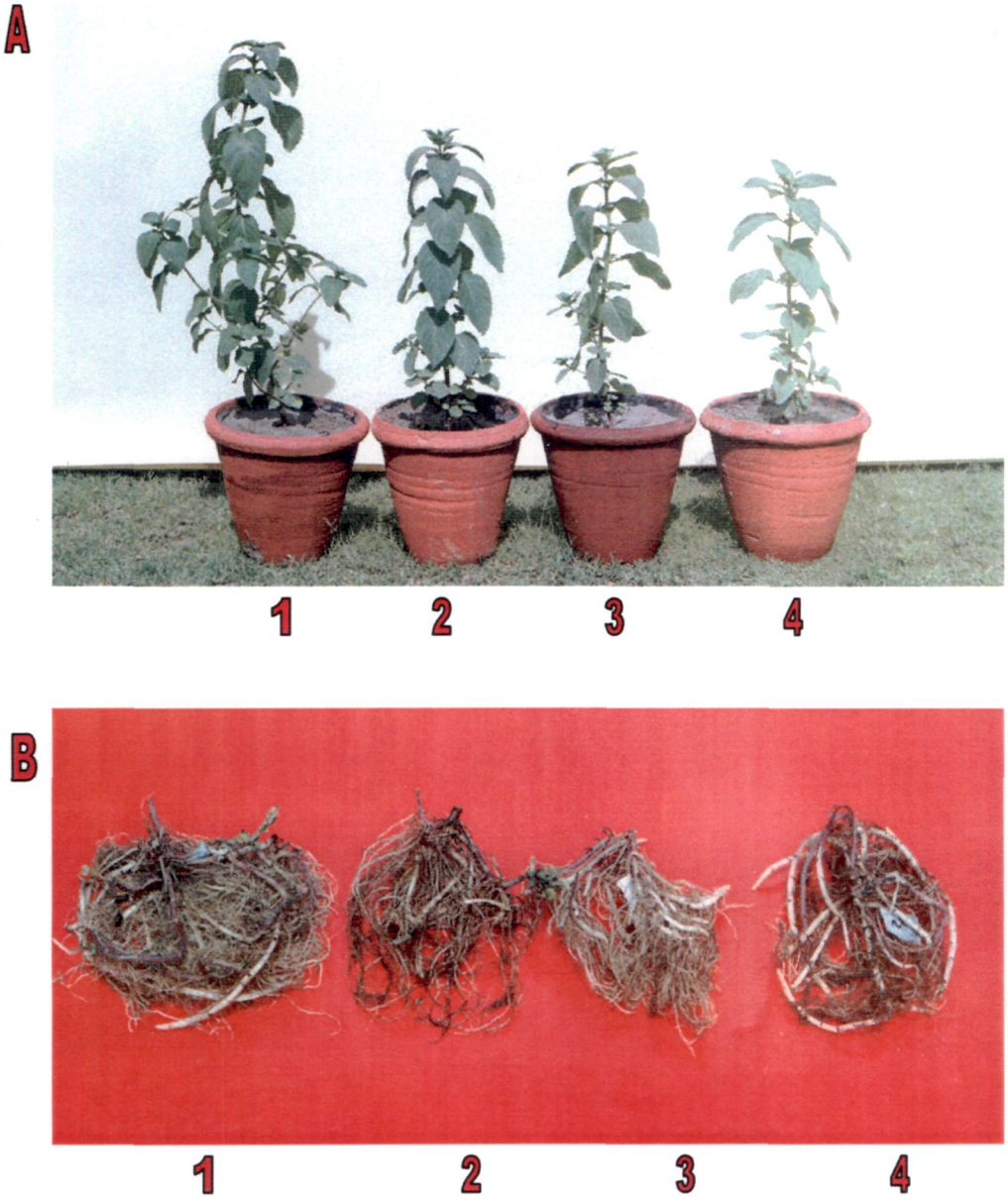


Plate 9: Effect of soil pH 6.0 on aerial growth (A) and roots and suckers development (B) of *M. arvensis* cv. Gomti.

1 = Uninoculated
2 = Inoculated with 3 g mycelium of *S. sclerotiorum*/pot
3 = Inoculated with 5000 J2 of *M. incognita*/pot
4 = Inoculated with 5000 J2 of *M. incognita* + 3 g mycelium of *S. sclerotiorum*/pot

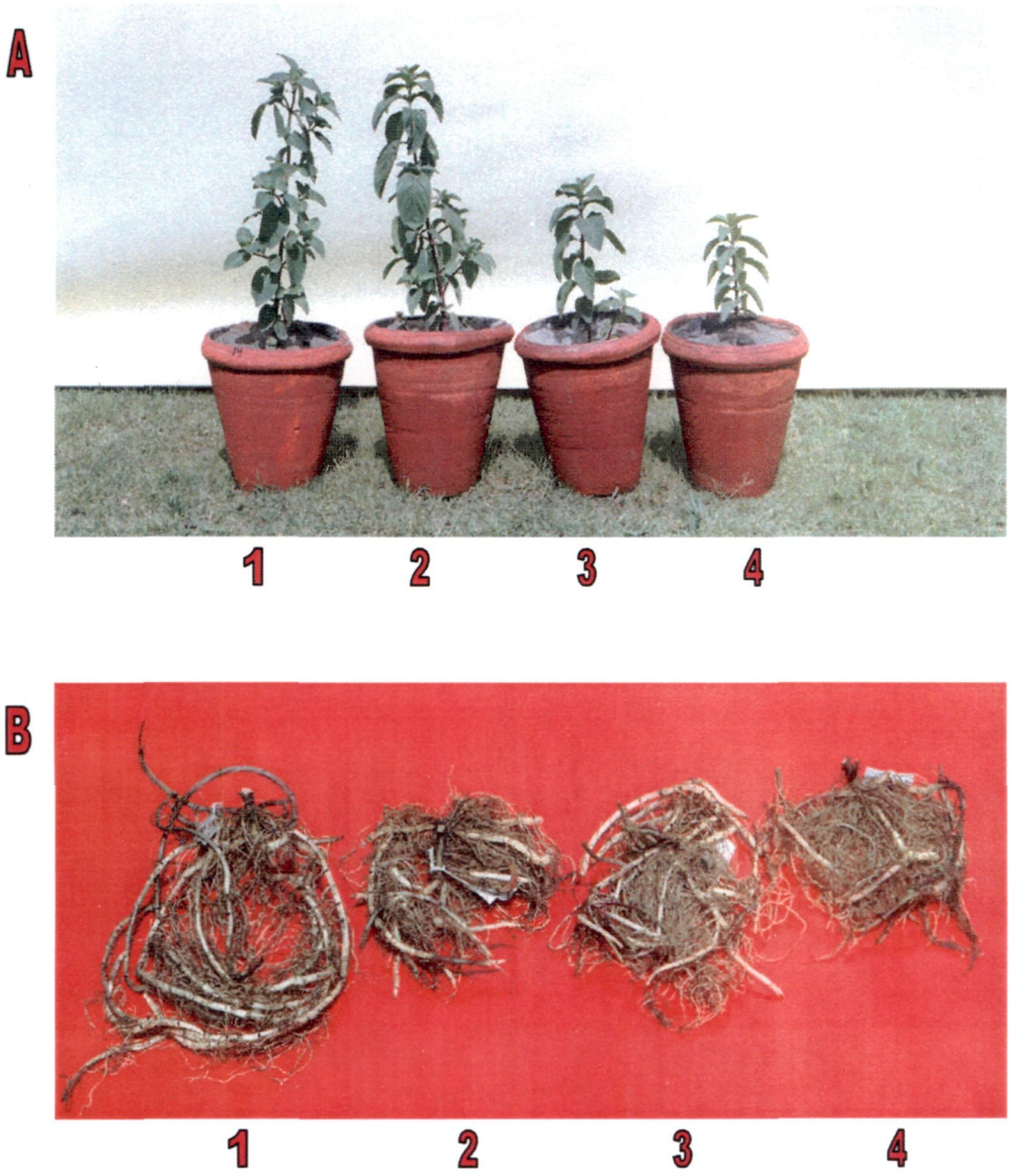


Plate 10: Effect of soil pH (7.5) on aerial growth (A) and roots and suckers development (B) of *M. arvensis* cv. Gomti.

1 = Uninoculated
2 = Inoculated with 3 g mycelium of *S. sclerotiorum*/pot
3 = Inoculated with 5000 J2 of *M. incognita*/pot
4 = Inoculated with 5000 J2 of *M. incognita* + 3 g mycelium of *S. sclerotiorum*/pot

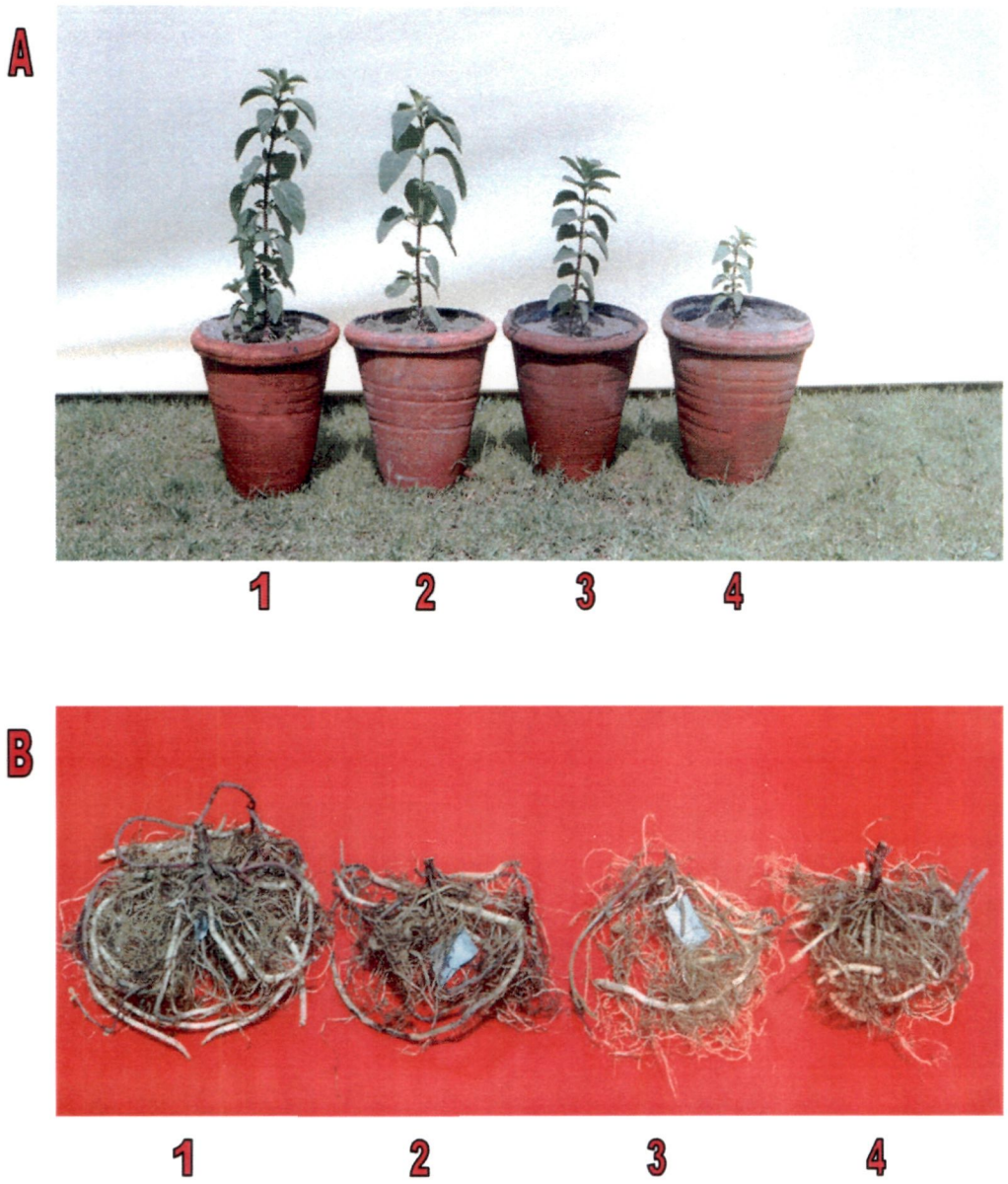


Plate 11: Effect of soil pH (9.0) on aerial growth (A) and roots and suckers development (B) of *M. arvensis* cv. Gomti.

1 = Uninoculated
2 = Inoculated with 3 g mycelium of *S. sclerotiorum*/pot
3 = Inoculated with 5000 J2 of *M. incognita*/pot
4 = Inoculated with 5000 J2 of *M. incognita* + 3 g mycelium of *S. sclerotiorum*/pot

Table 14: Effect of different pH levels on the reproduction of *Meloidogyne incognita* (5000 J2/5 kg soil), root-knot disease development and percent roots and suckers infection by *Sclerotinia sclerotiorum* (3 g mycelium/5 kg soil) on *Mentha arvensis* cv. Gomti.[a]

Treatments	Soil pH				Mean	L.S.D. $_{0.05}$	
	4.5	6.0	7.5	9.0		Pathogens/ pH	Interaction
Final nematode population (roots and suckers)							
Nematode alone	24500	36366	41405	45540	36952	537.15	474.29
Nematode + Fungus	13600	21920	24904	30000	22606		
Mean	9525	14571	16577	18885			
Final nematode population (soil)							
Nematode alone	26000	40000	52000	56000	43500	669.72	259.74
Nematode + Fungus	15000	26000	38000	42000	24500		
Mean	10250	16500	22500	24500			
Reproduction factor							
Nematode alone	10.10	15.27	18.68	20.31	16.10	0.67	0.36
Nematode + Fungus	5.72	9.58	12.58	14.40	10.60		
Mean	3.95	6.21	7.81	8.68			
[b]Root-knot index							
Nematode alone	1.15	1.60	1.75	2.25	1.69	0.35	0.70
Nematode + Fungus	0.62	1.00	1.37	1.60	1.15		
Mean	0.44	0.65	0.78	0.96			
[c]Roots and suckers infection							
Fungus alone	52.00	45.00	40.00	35.00	43.00	1.49	2.98
Nematode + Fungus	70.00	64.00	60.00	48.00	60.50		
Mean	30.50	27.20	25.00	20.70			

[a]Each value is an average of four replicates.
[b]Root-knot index: 0 = 0%, 1 = 1-25%, 2 = 26-50%, 3 = 51-75%, 4 = 76-100%.
[c]Percent roots and suckers infection by *S. sclerotiorum*.

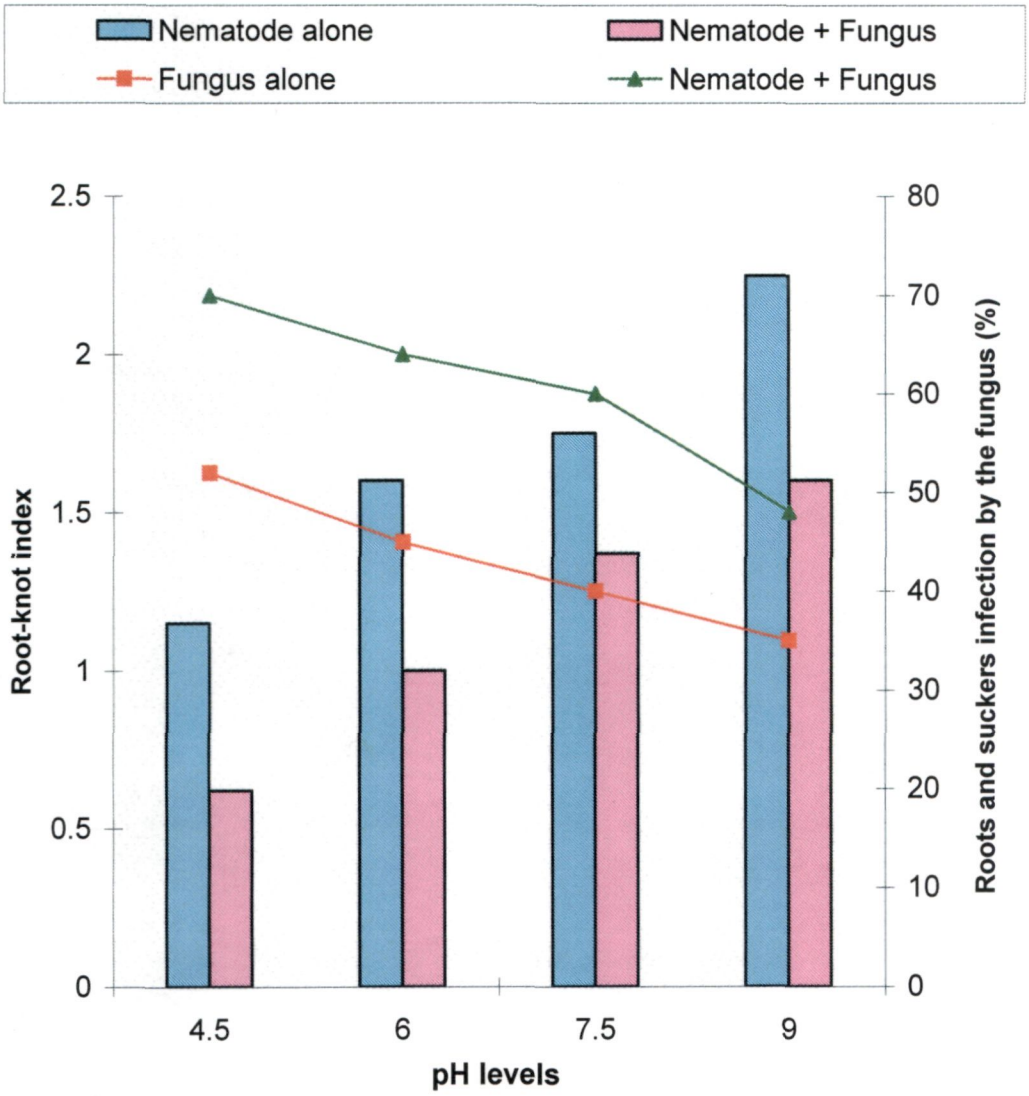


Fig. 11: Effect of different pH levels on disease development by *M. incognita* (5000 J2/5 kg soil) and *S. sclerotiorum* (3 g mycelium/ 5 kg soil) on *M. arvensis c* v. Gomti.

Root-knot index:- 0=0%, 1=1-25%, 2=26-50%, 3=51-75%, 4=76-100%

factor (20.31) and root-knot index (2.25) were observed at pH 9.0 in plants inoculated with nematode alone, whereas, the highest percent roots/suckers infection by fungus (70.00) was observed at pH 4.5 in plants inoculated with nematode and fungus simultaneously.

Biochemical data on *M. arvensis* cv. Gomti indicated that the chlorophyll a, chlorophyll b, total chlorophyll, total phenol and total sugar content of the uninoculated and inoculated plants with fungus alone increased with the increase in pH. The plants inoculated with nematode alone and nematode and fungus simultaneously showed increase in percent reduction of corresponding chemicals with increase in pH (Table 15).

Highest reduction in chlorophyll a (39.83 and 28.45%), chlorophyll b (40.58 and 28.98%), total chlorophyll (39.90 and 28.50%), total sugar (32.14 and 23.21%) and total phenol (37.60 and 27.20%) was observed at pH 9.0 in plants inoculated with nematode alone and nematode and fungus simultaneously, respectively. Similarly, highest reduction of biochemicals was 14.0, 13.3, 13.17, 13.04 and 13.20%, respectively observed at pH 4.5 in plants inoculated with fungus alone.

Analyses of data shows that the difference in chlorophyll a content of the leaves in the nematode and fungus free plants grown at pH 6.0 and 7.5 was non-significant ($P \leq 0.05$). Similarly, no difference in chlorophyll b content was observed between soil pH 7.5 and 9.0 in plants inoculated with nematode alone. In plants inoculated with nematode and fungus simultaneously, the differences in chlorophyll a, chlorophyll b and total chlorophyll between pH 7.5 and 9.0 were observed non-significant. The reduction in all chemicals in inoculated plants was significant ($P \leq 0.05$) as compared with uninoculated plants irrespective of pH levels.

DISCUSSION

Results indicated that the plants inoculated with nematode alone and nematode and fungus simultaneously showed maximum reduction in plant growth and oil yield at pH 9.0 followed by 7.5, 6.0 and 4.5, respectively. Hakim

Table 15: Effect of different pH levels on the biochemical changes in uninoculated, *Meloidogyne incognita* (5000 J2/5 kg soil) and/or *Sclerotinia sclerotiorum* (3 g mycelium/5 kg soil) inoculated plants of *Mentha arvensis* cv. Gomti.[a]

Parameters and treatments	Soil pH				Mean	L.S.D. $_{0.05}$	
	4.5	6.0	7.5	9.0		Pathogens/ pH	Interaction
Chlorophyll content (mg/g fresh leaves)							
Chlorophyll a							
Uninoculated control	1.07	1.17	1.19	1.23	1.16	0.01	0.03
Nematode alone	1.03 (3.74)[b]	0.98 (16.24)	0.92 (22.69)	0.88 (28.45)	0.95		
Fungus alone	0.92 (14.0)	1.03 (11.96)	1.09 (8.40)	1.16 (5.69)	1.05		
Nematode + Fungus	0.87 (18.7)	0.84 (28.20)	0.76 (36.13)	0.74 (39.83)	0.80		
Mean	0.97	1.00	0.99	1.00			
Chlorophyll b							
Uninoculated control	0.60	0.63	0.65	0.69	0.64	0.01	0.02
Nematode alone	0.54 (5.00)	0.52 (17.46)	0.49 (24.61)	0.49 (28.98)	0.51		
Fungus alone	0.52 (13.30)	0.56 (11.11)	0.59(9.23)	0.65 (5.80)	0.58		
Nematode + Fungus	0.48 (20.0)	0.45 (28.57)	0.41 (36.92)	0.41 (40.58)	0.44		
Mean	0.53	0.54	0.53	0.56			
Total chlorophyll							
Uninoculated control	1.67	1.79	1.85	1.93	1.81	0.02	0.04
Nematode alone	1.60 (4.19)	1.51 (15.64)	1.42 (23.24)	1.38 (28.50)	1.48		
Fungus alone	1.45 (13.17)	1.60 (10.60)	1.68 (9.19)	1.82 (5.70)	1.64		
Nematode + Fungus	1.35 (19.16)	1.30 (27.37)	1.18 (36.20)	1.16 (39.90)	1.25		
Mean	1.52	1.55	1.53	1.57			
Total phenol (mg/g fresh leaves)							
Uninoculated control	11.50	12.20	12.35	12.50	12.14	0.05	0.09
Nematode alone	11.00 (4.35)	10.68 (12.46)	9.50 (23.08)	9.10 (27.20)	10.07		
Fungus alone	10.00 (13.04)	10.85 (11.06)	11.10 (10.12)	11.35 (9.20)	10.82		
Nematode + Fungus	9.25 (19.56)	9.00 (26.22)	7.90 (36.00)	7.80 (37.60)	8.49		
Mean	10.44	10.68	10.21	10.19			
Total sugar (mg/g fresh leaves)							
Uninoculated control	12.50	13.15	13.90	14.00	13.39	0.9	1.9
Nematode alone	11.80 (5.60)	11.50 (12.55)	11.0 (20.86)	10.75 (23.21)	11.26		
Fungus alone	10.85 (13.20)	12.65 (11.41)	12.65 (9.00)	13.00 (7.14)	12.29		
Nematode + Fungus	10.12 (19.04)	9.85 (25.09)	9.65 (30.6)	9.50 (32.14)	9.78		
	11.32	11.79	11.80	11.81			

[a]Each value is an average of four replicates. [b]Figures in parentheses are percent reduction over uninoculated control.

et al. (1985), Volcy (1990) and Haseeb and Shukla (2000a) also observed reduction in plant growth with the increase in pH, in the plants inoculated with nematode alone, whereas, the plants inoculated with fungus alone showed maximum reduction in corresponding parameters at pH 4.5 followed by 6.0, 7.5 and 9.0 respectively. Similar results were observed on guar due to *S. rolfsii* (Singh and Gandhi, 1991) and on coriander due to *Protomyces macrosporus* (Tripathi *et al.*, 2002).

The observations recorded on the nematode multiplication and disease development indicated that the highest, reproduction rate and root-knot index were observed at pH 9.0 followed by 7.5, 6.0 and 4.5, respectively. Other workers also observed the similar relationship between the nematode multiplication and pH (Hakim *et al.*, 1985; Haseeb *et al.*, 1999a; Haseeb and Shukla, 2000a). However, Khan *et al.* (1997) observed that increase in soil salinity decreased the number of galls, number of eggs and egg masses. In another study Wallace (1966) found that *Meloidogyne* spp. survival, hatching and reproduction was between soil pH of 4.0-8.0, but maximum egg hatching was between the pH 6.4 to 7.0. However, the hatching was inhibited below pH 5.2.

The roots/suckers infection due to *S. sclerotiorum* was found to be highest at pH 4.5 followed by pH 6.0, 7.5 and 9.0, respectively. Several other reports also indicated that *Sclerotinia* spp. could tolerate a wide range of pH, however this fungus is well adapted to an acidic substrate or environment (Tanrikut and Vaughan, 1951; Khare *et al.,* 1981). Since the extracellular pectolytic and cellulolytic enzymes produced by *S. sclerotiorum* have optima pH below 5.0, therefore, the rapid disease development was observed at higher acidic pH (Echandi and Walker, 1957; Lumsden, 1969; Sharma *et al.*, 1984).

The reduction in plant growth parameters and oil yield was highest in plants inoculated with nematode and fungus followed by nematode alone and fungus alone, respectively, as compared to corresponding uninoculated plants, irrespective of soil pH. The highest nematode reproduction rate and root-knot index were observed in plants inoculated with nematode alone. Whereas, percent

roots and suckers infection by the fungus was highest in plants inoculated with fungus alone. These observations are in general agreement with the studies carried out on interactive effect of nematode and fungus on the plant growth and on pathogens by other workers (Haseeb, 1983; Sitaramaiah and Devi, 1990; Anwar and Khan, 2002).

The present study indicates that for the cultivation of *M. arvensis* cv. Gomti, farmers can be advised to adjust the pH towards acidic either temporarily or permanently by implementing appropriate means such as application of fertilizer or pyrite in their field. The lower pH will reduce the reproduction and activity of pathogens and will increase the nutrient uptake by plants thus resulting reduction in disease severity.

7. Effect of different soil types on disease development, nematode multiplication, growth, oil yield and biochemical changes in *M. incognita* and/or *S. sclerotiorum* inoculated plants of *M. arvensis* cv. Gomti

The environmental factors that most seriously effect the initiation and development of plant diseases are temperature and moisture. However, soil factors such as soil pH, soil structure, nutrient availability in soil, etc. also affect disease development through their influence on the growth and susceptibility of the host, on the multiplication and activity of the pathogen, and on the interaction of host and pathogen.

REVIEW OF LITERATURE

Plant-parasitic nematodes

Nematode population changes in response to the pressures and challenges imposed by external factors and so to develop a structure and show properties of dynamic equilibrium. It is difficult to relate population growth to such factors as rainfall, temperature, and soil type or host plant and to show precisely how each factor affects biological processes. In general, most of the information in literature deals in relation to soil type and densities of a particular nematode

species but rarely studied the soil variables and the development of the nematode (Norton, 1978).

The impact of soil texture on the activity of plant-parasitic nematodes and the associated effects on plant growth has been under study since the work of Bessey in 1911. He found that the severity of root–knot disease was decreased in susceptible plants grown in fine textured soil. Since then it has been established that the infestation and associated crop damage due to root-knot nematode is much greater in sandy soils than clay or heavy soils (Sasser, 1954; O'Bannon and Reynolds, 1961; Wallace, 1969; Barker and Olthof, 1976; Barker *et al.,* 1981; Shane and Barker, 1986; Wallace, 1989; Barker and Weeks, 1991; Koening *et al.*, 1996; Haseeb *et al.,* 1998; Sharma, 2001).

Ferris and Bernard (1971) studied the effect of four different soil types (Proctor silt loam, Drummer silty clay loam and Illiopolis silty clay loam) on population densities of *Helicotylenchus pseudorabustus, Tylenchorhynchus acutus, T. martini, Pratylenchus hexincius, P. scribneri, P. penetrans, P. projectus, P. neglectus* and *P. crenatus* in corn, soybean, wheat and forage crops rotation for droughty five years. They observed that the highest densities of *T. acutus* and *H. pseudorobustus* were found to be in silty clay loam, the silt loam soil favoured the multiplication of *P. projectus.* Interestingly the population of *Pratylenchus* species was low in all soil types in the rotated blocks.

Grandison and Wallace (1974) studied the effect of soil texture on the population of *P. thornei* in roots of *Trifolium fragiferum* and soil at eight different sites within 30km radius of Adelaide, Australia. The results indicated that nematode population increased with an increase in clay content of the soil and decreased with the increase in percent of sand. The highest number of *P. thornei* was obtained in roots as well as in soil when soil had 52% clay and 36% sand, whereas the lowest population was found in soil containing 6% clay and 93% sand.

Naganathan and Sivakumar (1975) evaluated the influence of three soil types (black sandy clay loam, red sandy loam and brown sandy loam) on the pathogenic potential of *Pratylenchus delattrei* on maize in a pot experiment. The

highest reduction in root and shoot weights was found in red sandy loam soil followed by brown sandy loam at Pi of 4 nematodes per 5 g soil. Black sandy clay loam, however, did not visualize significant yield reduction. The highest nematode population was recorded in brown sandy loam soil followed by black sandy clay loam and red sandy loam. They also concluded that there was a positive correlation between sand content of soil and nematode multiplication.

Zirakparvar *et al.* (1980) studied the influence of four soil types [Marshall silt loam (19% sand, 55% silt, 26% clay, pH 5.3), Clarion silt loam (16% sand, 58% silt, 26% clay, pH 6.2), Buckner coarse sand (86% sand, 9% silt, 5% clay, pH 6.3), and Haig silty clay loam (60% sand, 19% silt, 21% clay, pH 5.9)] and temperature on the population build up of *P. hexincisus* on corn and found that the nematode population was significantly higher in Buckner coarse sand than in other soil types at 30.5 °C. However, the highest final nematode population was noted in corn grown in Buckner coarse sand after three months of inoculation at 30.5 °C.

Prot and Van Gundy (1981) studied the vertical migration of *M. incognita* juveniles introduced at 20 cm from the tomato roots in fine natural soils (100% silica sand, 95% silica sand with 5% clay, 90% silica sand with 10% clay as a concentrated layer). In this study, they found that with the increase in percentage of clay and silt, the juveniles were capable to migrate 20 cm only and the penetration of J2 into the roots was also decreased. No migration occurred in silica sand without clay particles. When 5 or 10% clay were mixed to silica sand, 34 and 26 % respectively of juveniles were able to migrate 20 cm.

Handa *et al.* (1985) estimated the losses due to *Heterodera avenae* in barley crop grown in sandy and sandy loam soils and reported that the maximum losses were 91.8 and 87.2 % to fodder and grain respectively at an initial population of 22.4 eggs/g soil. However, the damage was noted to be much more in sandy soil as compared to sandy loam.

Windham and Barker (1986) conducted a field experiment at North Carolina to study the influence of different soil types [Fuquay sand (91% sand, 3% clay, 6% silt, pH 6.1), Norflok loamy sand (84% sand, 4% clay, 12% silt, pH

6.3), Portsmouth loamy sand (72% sand, 10% clay, 18% silt, pH 5.9), muck (58% sand, 9% clay, 33% silt, pH 5.0), Cecil sandy clay loam (53% sand, 29% clay, 18% silt, pH 6.7) and Cecil sandy clay (48% sand, 39% clay, 13% silt, pH 6.7)] on the damage potential of *M. incognita* on *Glycine max. M. incognita* reproduced readily on a susceptible soybean cultivar in most of the soil types with somewhat limited reproduction in muck soils. The relationship between initial population densities and yield varied among the soil types and nematode populations. Highest reduction in yield was found in sandy and muck soil types, whereas lowest reduction in yield was obtained in clay soil.

Nakasono *et al.* (1989) evaluated the effect of *M. incognita* on growth of tomato plants grown in different soil types (andosol-1, andosol-2, red-yellow soil and gray low land soil). They found that average fresh weight of the above ground parts of tomato plants in the nematode free plots was recorded up to 196 g, 203 g, 218 g and 246 g for andosol-1, gray low land soil, red-yellow soil and andosol-2 respectively. Though, tolerance limits in the weight of above ground parts were noted to be 0.05, 0.1, 1.0 and 2.0 J2/ml soil for andosol-1, andosol-2, gray low land soil and red-yellow soil, respectively. The fresh root weight was, however, increased with the increase in inoculum levels in all the soil types. Gall indices of tomato roots did not differ among the soil types, but the degree of occurrence of brownish and necrotic symptoms on root was greater in the gray low land soil regardless of inoculum levels.

Nakasono *et al.* (1990) while investigating the effect of six initial inoculum densities of *M. incognita* (0-33,000/pot) on the growth of tomato in different soil types (andosol, sirasu, sand-dune regosol and gray low land soil), found a significant decrease in plant height in all soil types except gray low land soil. The highest plant top weight (shoot + fruit) was recorded in sirasu soil followed by gray low land, andosol, and sand dune regosol respectively. Root weight was increased with the increase in inoculum levels in each soil. However, chemical properties of the soils did not show any difference in plant growth due to nematode infection.

Barker and Weeks (1991) evaluated the effect of six soil types [Cecil sandy clay (47.9% sand, 38.9% clay, 13.2% silt), Cecil sandy clay loam (52.8% sand, 28.7% clay, 18.5% silt), Fuquay sand (91.0% sand, 2.5%, clay, 6.5% silt), Muck (58.1% sand, 8.7% clay, 33.2% silt). Norfolk loamy sand (83.5% sand, 4.0% clay, 12.5% silt), Portsmouth loamy sand (71.1% sand, 10.0% clay, 18.3% silt)] and four inoculum levels of *M. incognita* (0, 1250, 5000, 20000 eggs/500 cm^3 soil) on nematode reproduction, yield and quality of tobacco under field conditions. Tobacco yield was significantly reduced at all initial inoculum levels in each soil type, but the highest damage (93%) occurred in Cecil sandy clay and Muck soil at Pi of 20,000 eggs/500 g soil, followed by 89% in Portsmouth loamy sand, 79% in Norfolk loamy sand, 78% in Fuquay sand, and 60% in Cecil sandy clay loam. Interestingly, the highest yield was recorded in Norfolk loamy sand followed by Portsmouth loamy sand, Muck, Cecil sandy clay loam, Fuquay sand and Cecil sandy clay respectively. *M. incognita* was found to increase the sugar content in tobacco leaf at low initial inoculum densities, whereas, higher initial population densities significantly decreased the sugar content. All levels of nematode population reduced the reducing sugar synthesis in tobacco leaves. Soil types had a marked effect on reducing sugar of the tobacco leaves. Tobacco from Portsmouth loamy sand had the lowest sugar content but its differences varied among other soils. The presence of *M. incognita* had a negative impact on nicotine content of the cured tobacco leaf in all soil types irrespective of inoculum levels. Higher reproduction rate of *M. incognita* was found in Norfolk loamy sand and the Portsmouth loamy sand after 10-12 weeks of planting at lower inoculum densities than in clay and organic soils.

Anwar and Khan (1992) studied the influence of different soil types (clay, sandy clay loam, silt loam, sandy loam, and sand) on the reproduction of *P. zeae* on corn cvs. Sultan and Sunahri in greenhouse conditions. Clay soil was found to be the most suitable for the development of infection and reproduction of *P. zeae* on both cultivars of corn followed by silt loam and sandy clay loam respectively. Highest multiplication of the nematode was found in cv. Sultan than Sunahri in all the soil types.

Shahina and Maqbool (1993) conducted a greenhouse experiment to study the effect of different soil types (sand, clay, sandy loam, sand + clay and sand + sandy loam) on *Heterodera cynodontis* in various crop plants and found that maximum reproduction of *H. cynodontis* occurred in sand + sandy loam soil.

Koenning *et al.* (1996) studied the effects of soil type and initial inoculum density on the reproductive and damage potentials of *M. incognita* and *R. reniformis* on cotton. They reported the reproduction of *M. incognita* was greater in coarse-textured soil than in fine textured soil, whereas, reproduction factor of *R. reniformis* was greatest in a Portsmouth loamy sand intermediate percentage of clay plus silt. Population densities of *M. incognita* were inversely proportional to the percentage of silt and clay soil. Both races (3 and 4) of *M. incognita* suppressed the yield of seed cotton grown in all types of soil tested.

Shukla *et al.* (1998) determined the pathogenic potential of *P. thornei* on *M. citrata* grown in different soil types. They observed that *P. thornei* reproduced best (Rf = 8.49) in sandy clay loam soil followed by loamy sand (Rf = 7.80) and sandy loam (Rf = 6.41), respectively in suckers/roots inoculated with 12,500 nematode/pot. The greatest reduction in growth and oil yield of *M. citrata* occurred in sandy clay loam followed by loamy sand and sandy loam at initial inoculum (Pi) levels of 12,500 and 25,000 nematodes/pot. In the absence of nematodes, the greatest plant growth and oil yields were in sandy loam soil followed by sandy clay loam and loamy sand.

Haseeb *et al.* (1999b) studied the effect of different soil types (loamy sand, sandy loam, sandy clay loam and silty loam) on the growth and oil yield of *O. canum* and reproduction of *M. incognita*. Highest growth and oil yield of plants in absence of nematode was observed in sandy loam (SL) followed by sandy clay loam (SCL), loamy sand (LS) and silty loam (SIL), respectively. Reduction in shoot growth was highest in LS followed by SCL, SL and SIL respectively. Increase/decrease in root fresh and dry weights was found to be dependent upon the severity of root galling. Oil content in fresh shoot was observed highest in plants grown in SIL followed by SCL, LS and SL respectively. Final nematode population was highest in SL followed by SCL, LS

and SIL respectively. Root-knot index was observed highest in LS followed by SCL, SL and SIL respectively. All the soil types influenced root-knot index but there was no difference in Pf between SCL and LS

Shukla and Haseeb (1999) determined the effect of three soil types and three population levels of *P. thornei* on the plant growth, oil yield, chlorophyll, total sugar and phenol content in leaves of *M. spicata* cv. MSS-5 in a glass house pot experiment. Highest reproduction rate of *P. thornei* was 7.43 in plants inoculated with 12,500 nematodes per pot grown in sandy clay loam and lowest (2.74) in plants inoculated with 25,000 *P. thornei* per pot grown in sandy loam soil. Lowest plant length, fresh and dry weight, oil yield, chlorophyll content, photosynthetic rate, total sugar and phenol content in leaves found in sandy clay loam followed by loamy sand and sandy loam, respectively at both initial inoculum densities as compared to uninoculated controls. Reduction in all the above test parameters due to nematode inoculations was highly significant. Soil type always affected plant variables in the absence of nematodes, but had less effect on plants inoculated with *P. thornei.*

Sharma (2001) determined the effect of *M. incognita* on French bean grown in different types of soil. French bean grown in loamy sand soil was least infested with *M. incognita* compared with sandy loam. Soil with highest organic contents supported the minimum multiplication of the nematode. Coarse-textured soils were found best for the multiplication of the nematode.

Phytopathogenic fungi

Soil factors such as pH, structure of soil, minerals and nutrients in soil affect the disease development through their influence on the growth and susceptibility of the host, on multiplication, growth and activity of the pathogen, or on the interaction of host and pathogen (Agrios, 2000).

Papavizas (1968) reported that the maximum saprophytic activity of *R. solani* was found in the medium and coarse sand fractions of a naturally infested sandy loam or loamy sand. Whereas, its little activity was noted in the fine sand, silt of clay fractions.

Shukla (1972) studied the relation of *Ozonium texanum* and soil composition on guar and found that the plants grown in sand did not show pre-emergence mortality but it registered 100% mortality in post-emergence phase. The pots containing only clay exhibited maximum (44.13%) pre-emergence mortality and minimum (17.10%) post-emergence mortality. In the intermediate lots with decreasing proportions of clay and increasing proportions of sand, the pre-emergence mortality was gradually decreased, but on the other hand the post-emergence mortality was increased.

Merriman (1976) reported that sclerotia of *S. sclerotiorum* survived better in a sandy clay loam soil at pH 6.0 than in a sandy loam at soil pH 8.7. Naiki and Kanoh (1978) observed that the disease incidence due to *R. solani* on spinach was found to be less in light clay soil than in clay loam or loam soil. Johnson *et al.* (1978) reported that the *R. solani* causing post-emergence seedling disease of cotton was more frequently isolated from fine sandy loam soil than either from heavier loam or a silty loam.

Lewis (1979) studied the influence of soil texture on survival and saprophytic activity of *R. solani* and reported that survival of the fungus in pre-colonized table beet seed was greater in a light sandy loam soil than heavy silty clay loam. In natural soils of different texture, this activity was maintained longer in light sandy loam soils than light loamy-sand or loam.

Singh and Singh (1983) studied the effect of soil types, soil moisture and depth of soil on carpogenic germination of *S. sclerotiorum.* They found that the apothecial formation of *S. sclerotiorum* was more in sandy soils than in clay soils under moist conditions. Mitov (1987) reported that the sclerotia of *S. sclerotiorum* causing sunflower wilt remained viable for 6 months under the normal weather conditions in alluvial meadow soil at depths of 0-30 cm.

Das *et al.* (1987) studied the effect of certain soil types *in vitro* on the growth of *S. rolfsii* causing stem rot of groundnut and found that irrespective of pH levels and energy sources, the growth rate of the fungus was relatively better in light soils than heavy soils with more of clay. Only two textural soil classes,

viz clay loam (pH 6.8) and sandy loam (pH 6.3) supported the sclerotial formation of the fungus.

Mitchell and Wheeler (1990) studied the factors affecting the production of apothecia and longevity of sclerotia of *S. sclerotiorum*. They reported that overall production of apothecia was not affected by soil type but fewer numbers of sclerotia were recovered from the upper layers of soil with higher clay content.

Singh *et al.* (1991) reported that the physico-chemical properties and ion exchange capacity of soil samples influenced apothecial germination of sclerotia of *S. sclerotiorum*. Carpogenic germination was reduced in those soils with higher levels of organic carbon, whereas, there was no effect of pH on germination. The percentage of germination of apothecia was observed to increase with increasing amounts of sand in the soil samples and was reduced with increasing amounts of silt and clay.

Hossain *et al.* (1992) studied the influence of textural classes of soil (coarse sand, sandy loam, silty loam, clay loam and loam) on the incidence and development of diseases caused by *F. oxysporum, S. rolfsii* and *M. incognita* individually. They reported that the sandy loam and coarse sand were the best soil types for the development of diseases caused by both *F. oxysporum* and *R. rolfsii.* Whereas, fine textured soils were found unsuitable for these pathogens. The severity of root-knot development and the population of *M. incognita* was highest in coarse sand followed by sandy loam soil, and the activity of *M. incognita* was low in silty loam, loam and clay loam soil.

Fravel *et al.* (1996) studied the effect of soil types (Galestown gravelly loamy sand, Hatboro loamy sand and Hawaii clay loam soil) on the proliferation of *F. oxysporum* f. sp. *erythroxyli* isolate EN4-FT. They reported that metric potential and soil type significantly affected proliferation of the pathogen into the soils. The maximum proliferation of the fungus was observed in Hawaii clay loam soil and was lowest in Hatboro loamy sand soil.

Twengstrom *et al.* (1998) conducted field experiments to study the influence of different irrigation regimes and of soil types on apothecial production of *S. sclerotiorum.* They reported that high irrigation level caused more abundant apothecia production in the sand than the loam, while at low irrigation level more apothecia were produced in the loam than in the sand.

Sati and Sinha (1999) studied the survival of *R. solani* under different soil types and noted that the survival of *R. solani* was more in sandy loam than in clay loam or Bhabher soils. The fungus survived in plant debris up to 33.3 and 40% in clay loam and sandy loam, respectively after 150 days of inoculation. Sclerotia placed in sandy loam, clay loam and Bhabher soils, survived up to 36.7, 33.7 and 30.0%, respectively after 330 days.

Srivastava and Kamthan (2002) studied the effect of different soil types on the incidence of wilt of chickpea caused by *F. oxysporum* f. sp. *ciceri.* They reported that the black soil supported highest wilt incidence (75.5%), while in sandy loam, red soil, and clay soil wilt incidence was noted 64.4, 59.9, and 46.6% respectively.

MATERIALS AND METHODS

7.1 Collection and mechanical analysis of soil

Collection of soil was done from different cultivated fields around Aligarh. Mechanical analysis of these soils was done by the International pipette method (Pipper, 1950). After the analysis, four soil types, namely sandy clay (46% sand, 9% silt, 45% clay, pH 7.5), sandy clay loam (50% sand, 16% silt, 34% clay, pH 7.0), sandy loam (70% sand, 22% silt, 8% clay, pH 7.5) and loamy sand (80% sand, 11% silt, 9% clay, pH 7.5) was selected for the experiment.

7.2 Transplanting, inoculation and recording of data

The selected soils were autoclaved separately and were filled in 30-cm-diameter clay pots. In each pot, a single sucker was transplanted. For each soil type, inoculation of *M. incognita* and *S. sclerotiorum* was done according to the scheme described in 5.3. There were four replicates for each treatment. The

experiment was laidout as a completely randomized block design. Transplanting, inoculation and recording of data was done in the same manner as described earlier in 2 and 4.

RESULTS

The effect of different soil types on plant growth of *M. arvensis* cv. Gomti was significant, both in absence and presence of *M. incognita* and *S. sclerotiorum*. Poorest plant growth and oil yield was observed in loamy sand soil followed by sandy loam, sandy clay loam and sandy clay, respectively, as compared to uninoculated plants (Table 16; Fig. 12 and 13; Plate 12-15).

The highest reduction in all plant growth parameters and oil yield was observed in plants inoculated with the nematode and fungus simultaneously followed by nematode alone and fungus alone respectively, irrespective of soil type. The reduction in shoot height, shoot-roots/suckers dry weights and oil yield was highest (33.3, 50.4, 53.9 and 34.3%, respectively) in plants grown in loamy sand soil and inoculated with nematode and fungus simultaneously. Whereas, lowest reduction in plant growth was observed (2.8, 6.9, 7.1 and 3.2%, respectively) in loamy sand soil inoculated with fungus alone.

Analyses of data indicated that effect of different soil types and pathogens on different plant growth parameters were mostly significant. However, shoot height, shoot fresh and dry weights had no differences between nematode alone and fungus alone inoculated plants, grown in sandy clay soil. No differences ($P \leq 0.05$) were found in oil yield of the plants inoculated with fungus alone and grown in different soil types. Similarly, non-significant differences were observed in oil yield of plants grown in sandy loam and loamy sand and inoculated with nematode alone and nematode and fungus simultaneously, and between sandy clay and sandy clay loam in plants inoculated with nematode alone.

The different soil types had a significant effect on the *M. incognita* multiplication and percent roots/suckers infection by *S. sclerotiorum* (Table 17; Fig. 14). The highest final nematode population (roots/suckers and soil),

Table 16: Effect of different soil types on the growth and oil yield of uninoculated, *Meloidogyne incognita* (5000 J2/5 kg soil) and/or *Sclerotinia sclerotiorum* (3 g mycelium/5 kg soil) inoculated plants of *Mentha arvensis* cv. Gomti.[a]

Parameters and treatments	Soil types				Mean	L.S.D.$_{0.05}$	
	Sandy clay	Sandy clay loam	Sandy loam	Loamy sand		Pathogens/ Soil types	Interaction
Shoot height (cm)							
Uninoculated control	75.5	71.4	66.0	60.0	68.2	1.9	3.9
Nematode alone	72.0 (4.6)[b]	64.5 (9.7)	56.5 (17.4)	48.5 (19.2)	60.4		
Fungus alone	73.5 (2.8)	67.6 (5.3)	59.0 (10.6)	52.0 (13.3)	63.0		
Nematode + Fungus	70.0 (7.3)	60.0 (16.0)	45.5 (31.1)	40.0 (33.3)	53.9		
Mean	72.7	65.9	56.7	50.1			
Shoot fresh weight (g)							
Uninoculated control	195.2	165.0	132.4	120.0	153.2	5.0	4.0
Nematode alone	180.5 (7.5)	137.0 (17.0)	100.0 (24.5)	87.0 (27.6)	126.1		
Fungus alone	184.0 (5.7)	145.5 (11.8)	108.0 (18.0)	95.2 (21.0)	133.2		
Nematode + Fungus	167.0 (14.4)	116.5 (29.4)	72.0 (45.6)	57.5 (52.2)	102.7		
Mean	181.7	141.0	102.6	90.0			
Roots and suckers fresh weight (g)							
Uninoculated control	146.0	134.5	123.0	112.0	128.9	3.0	6.0
Nematode alone	133.5 (8.6)	111.0 (17.5)	91.0 (26.0)	78.0 (30.3)	103.2		
Fungus alone	136.7 (6.3)	118.0 (12.3)	99.5 (19.1)	86.8 (22.5)	110.2		
Nematode + Fungus	122.6 (16.0)	94.0 (30.1)	63.0 (48.8)	52.0 (53.6)	82.4		
Mean	134.7	114.4	93.8	82.2			
Shoot dry weight (g)							
Uninoculated control	46.5	39.5	32.0	29.0	36.7	1.4	0.9
Nematode alone	42.5 (8.6)	32.3 (18.0)	24.0 (25.0)	20.8 (28.3)	29.9		
Fungus alone	43.3 (6.9)	34.5 (12.6)	26.0 (18.7)	22.7 (21.7)	31.6		
Nematode + Fungus	39.5 (15.0)	27.5 (30.4)	17.1 (46.6)	13.5 (53.4)	24.2		
Mean	42.9	33.5	24.6	21.5			
Roots and suckers dry weight (g)							
Uninoculated control	28.3	26.0	23.5	21.7	24.9	0.6	1.0
Nematode alone	25.7 (9.2)	21.3 (18.1)	17.4 (25.9)	15.0 (30.9)	19.8		
Fungus alone	26.3 (7.1)	22.6 (13.1)	18.7 (20.4)	16.6 (23.5)	21.0		
Nematode + Fungus	23.5 (17.0)	18.0 (30.8)	12.1 (48.5)	10.0 (53.9)	15.7		
Mean	25.9	22.0	17.8	15.8			
Oil yield (ml/100 g fresh herb)							
Uninoculated control	0.62	0.64	0.68	0.70	0.66	0.01	0.02
Nematode alone	0.59 (4.8)	0.58 (9.4)	0.55 (19.1)	0.54 (21.0)	0.56		
Fungus alone	0.60 (3.2)	0.61 (4.7)	0.62 (8.8)	0.62 (11.4)	0.61		
Nematode + Fungus	0.56 (9.7)	0.54 (15.6)	0.47 (30.9)	0.46 (34.3)	0.51		
Mean	0.59	0.59	0.58	0.58			

[a]Each value is an average of four replicates. [b]Figures in parentheses are percent reduction over uninoculated control.

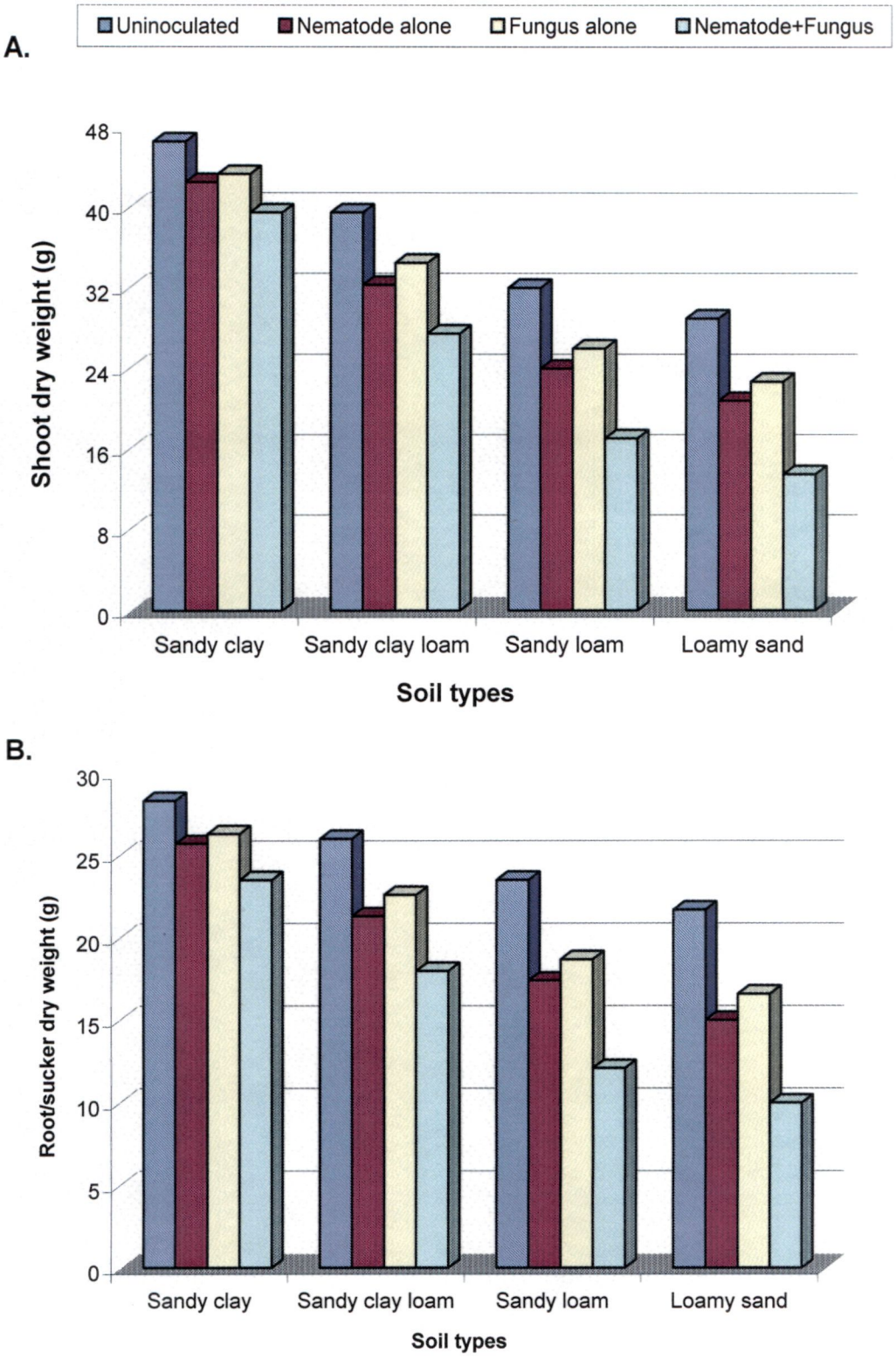


Fig.12: Effect of different soil types on shoot (A) and root/sucker (B) dry weights of uninoculated and inoculated (5000 J2 of *M. incognita* and/or 3 g mycelium of *S. sclerotiorum* /5 kg soil) plants of *M. arvensis* cv. Gomti.

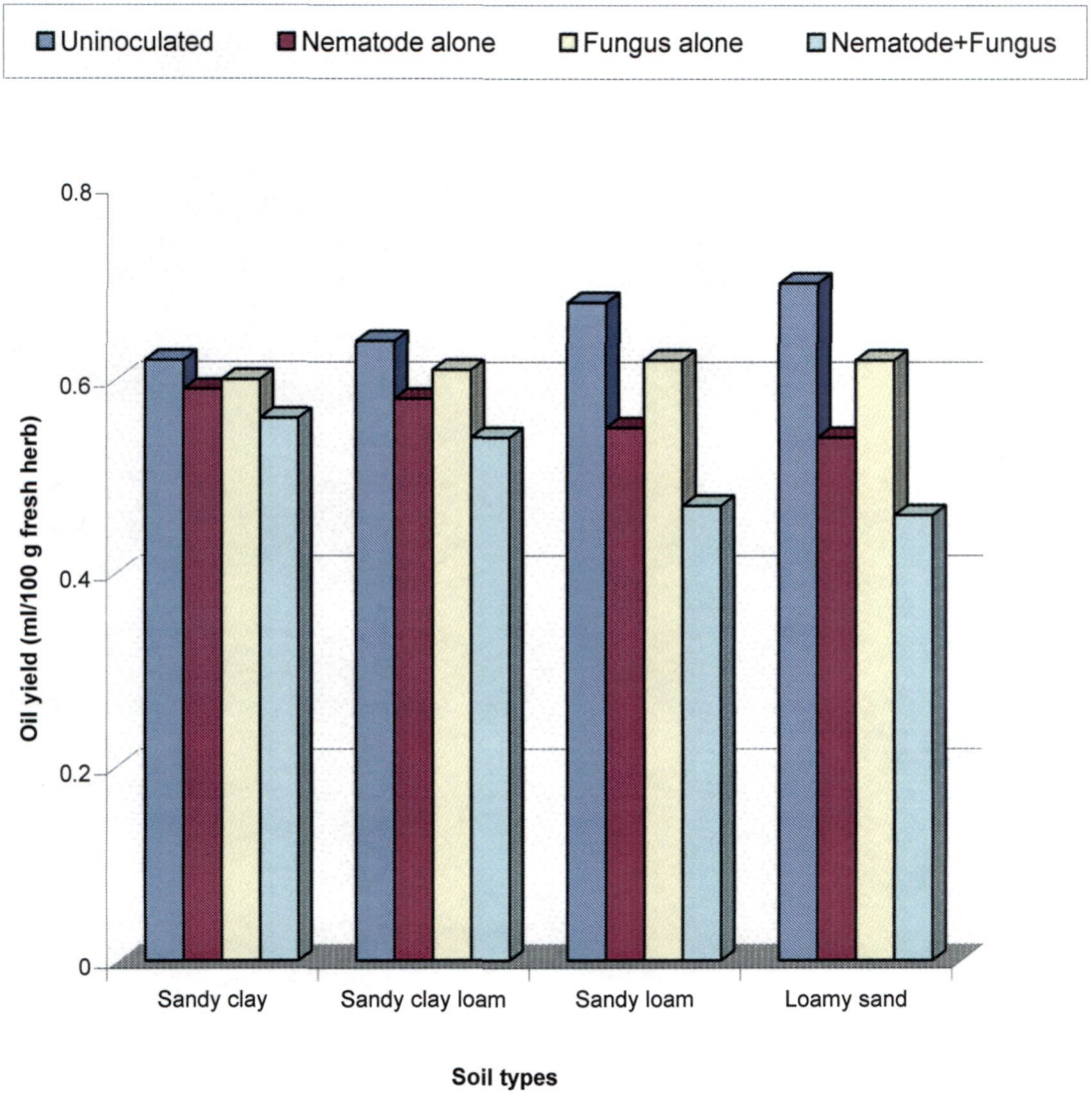


Fig.13: Effect of different soil types on oil yield of uninoculated and inoculated (5000 J2 of *M. incognita* and/or 3 g mycelium of *S. sclerotiorum* /5 kg soil) plants of *M. arvensis* cv. Gomti.

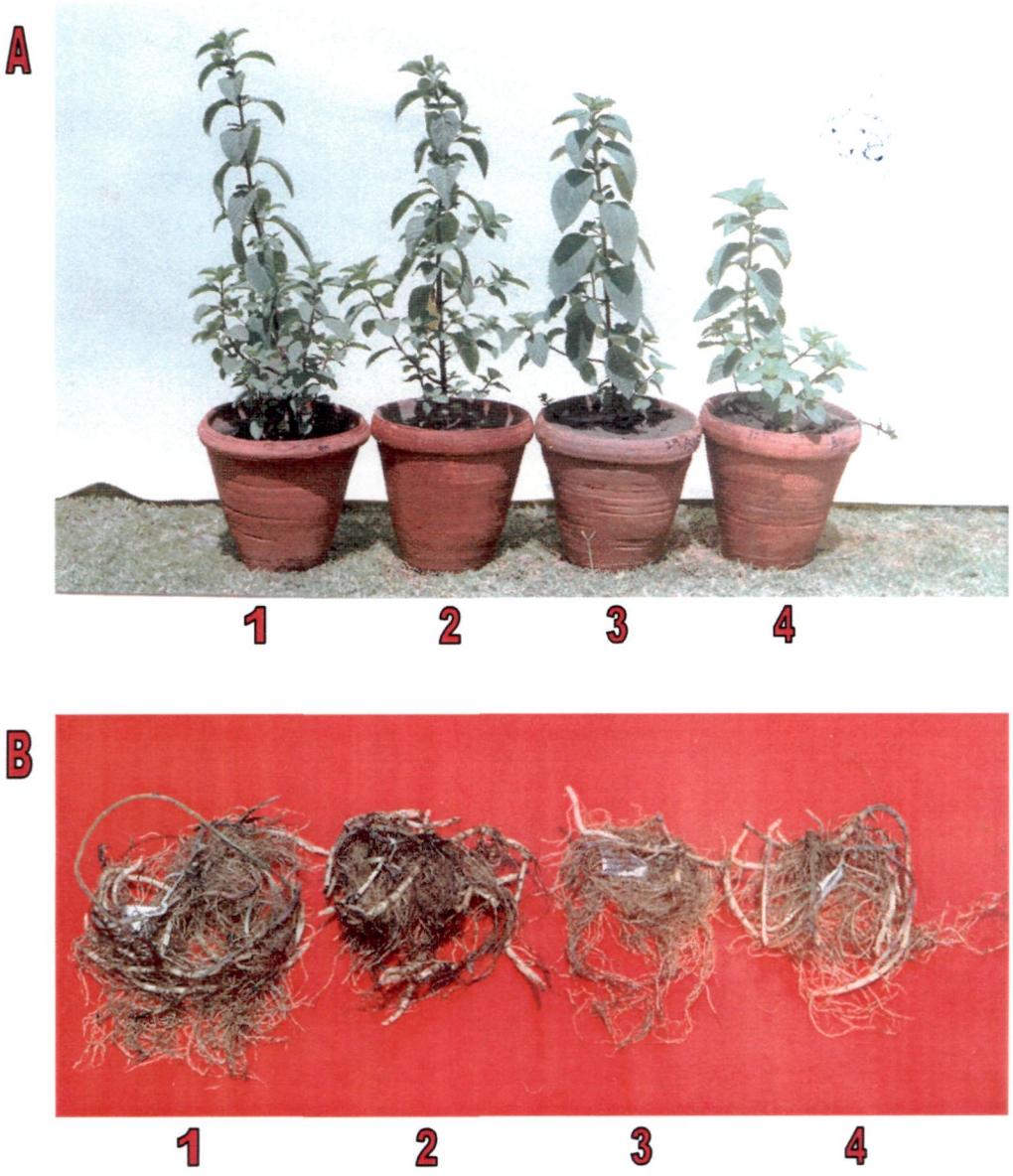


Plate 12: Effect of sandy clay soil on aerial growth (A) and roots and suckers development (B) of *M. arvensis* cv. Gomti.

1 = Uninoculated
2 = Inoculated with 3 g mycelium of *S. sclerotiorum*/pot
3 = Inoculated with 5000 J2 of *M. incognita*/pot
4 = Inoculated with 5000 J2 of *M. incognita* + 3 g mycelium of *S. sclerotiorum*/pot

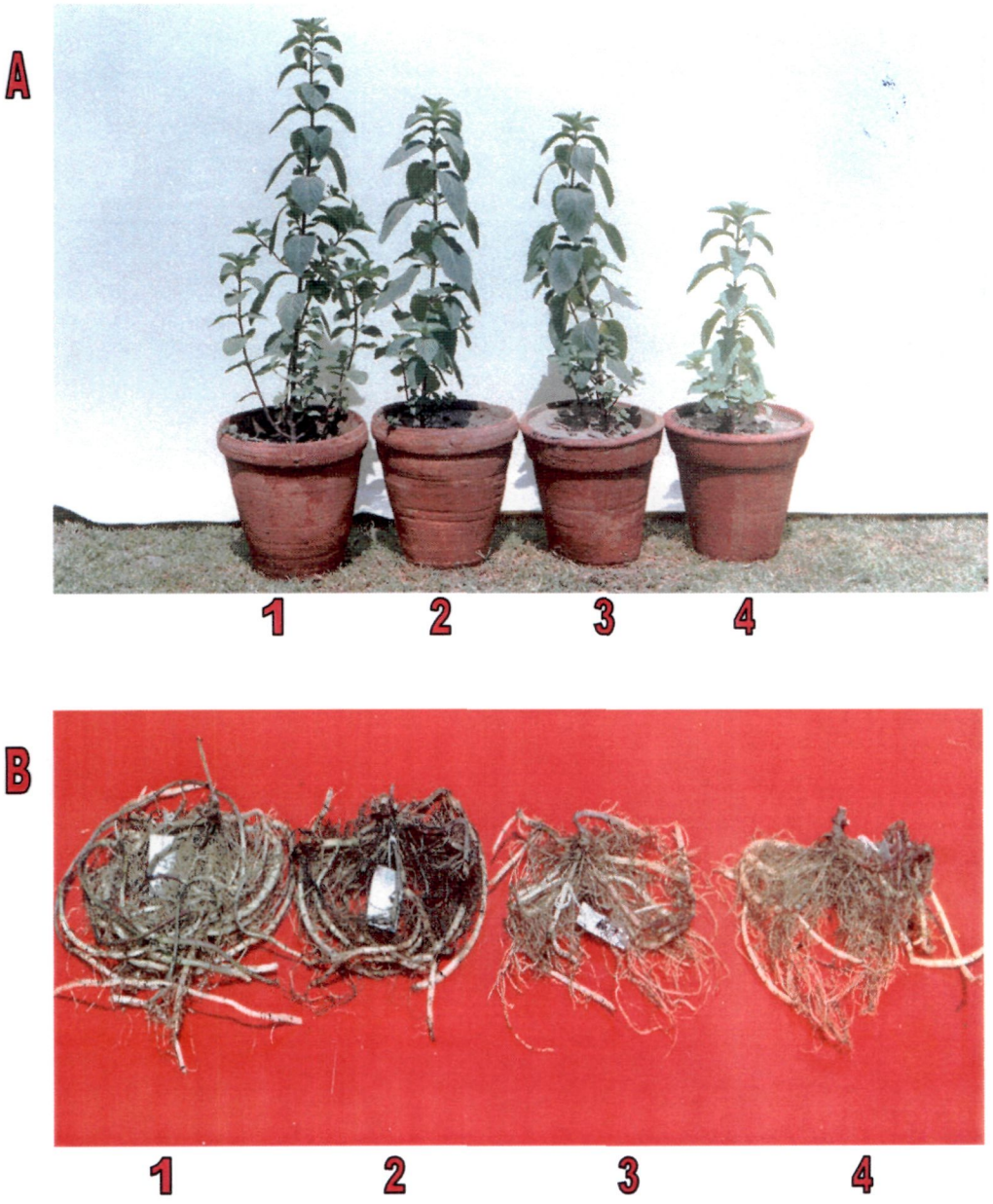


Plate 13: Effect of sandy clay loam soil on aerial growth (A) and roots and suckers development (B) of *M. arvensis* cv. Gomti.

1 = Uninoculated
2 = Inoculated with 3 g mycelium of *S. sclerotiorum*/pot
3 = Inoculated with 5000 J2 of *M. incognita*/pot
4 = Inoculated with 5000 J2 of *M. incognita* + 3 g mycelium of *S. sclerotiorum*/pot

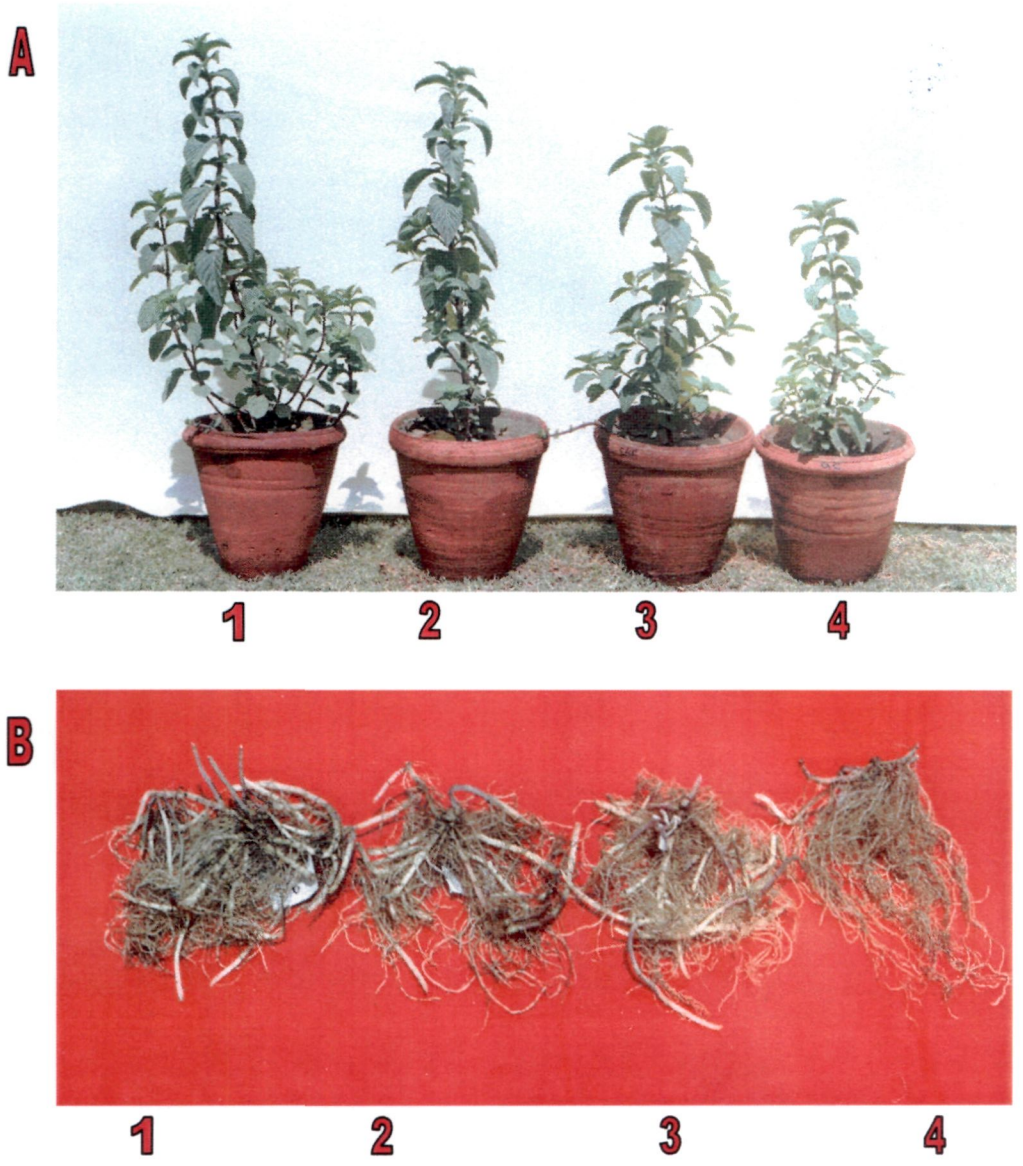


Plate 14: Effect of sandy loam soil on aerial growth (A) and roots and suckers development (B) of *M. arvensis* cv. Gomti.

1 = Uninoculated
2 = Inoculated with 3 g mycelium of *S. sclerotiorum*/pot
3 = Inoculated with 5000 J2 of *M. incognita*/pot
4 = Inoculated with 5000 J2 of *M. incognita* + 3 g mycelium of *S. sclerotiorum*/pot

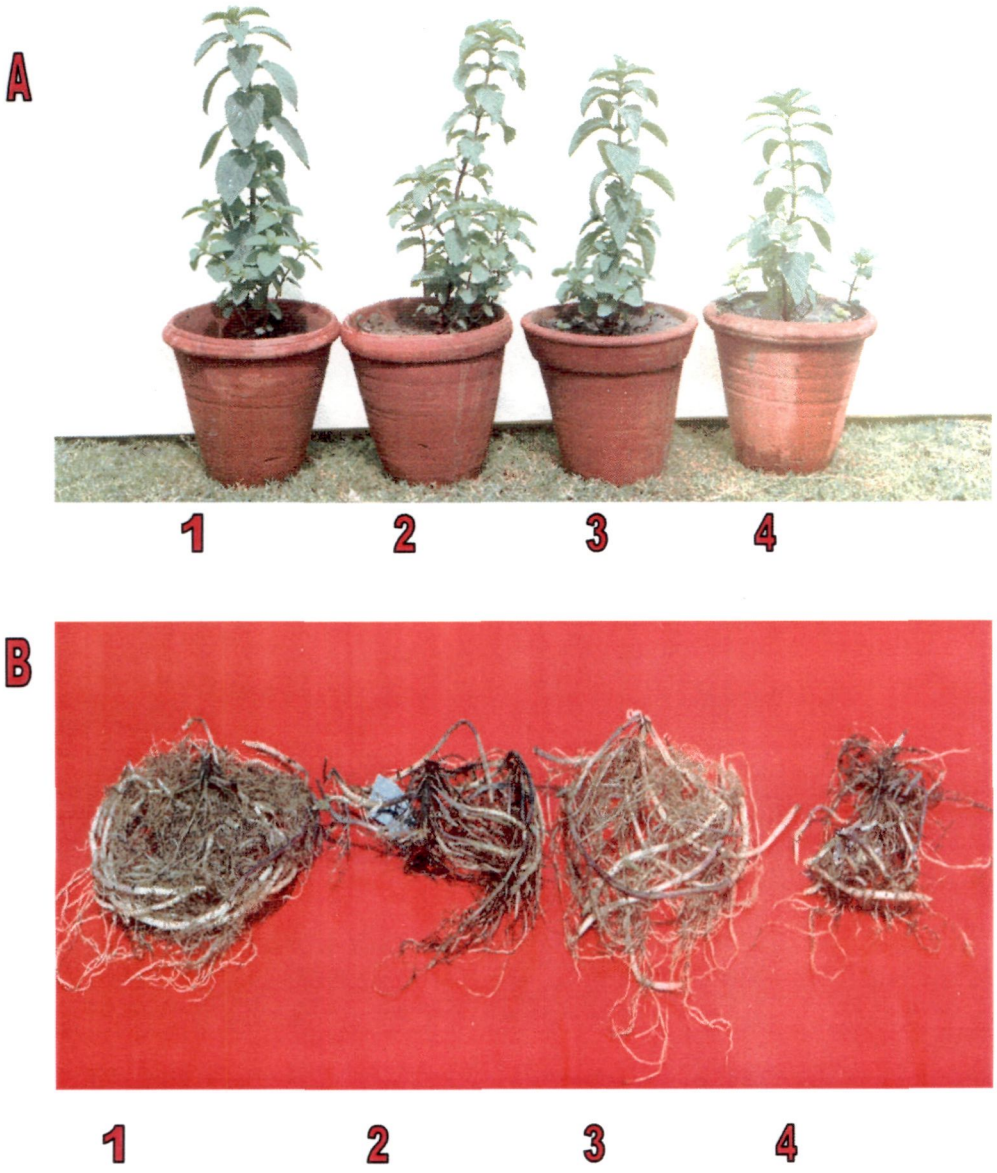


Plate 15: Effect of loamy sand soil on aerial growth (A) and roots and suckers development (B) of *M. arvensis* cv. Gomti.

1 = Uninoculated
2 = Inoculated with 3 g mycelium of *S. sclerotiorum*/pot
3 = Inoculated with 5000 J2 of *M. incognita*/pot
4 = Inoculated with 5000 J2 of *M. incognita* + 3 g mycelium of *S. sclerotiorum*/pot

Table 17: Effect of different soil types on the reproduction of *Meloidogyne incognita* (5000 J2/5 kg soil), root-knot disease development and percent roots and suckers infection by *Sclerotinia sclerotiorum* (3 g mycelium/5 kg soil) on *Mentha arvensis* cv. Gomti.[a]

Treatments	Soil types				Mean	L.S.D. $_{0.05}$	
	Sandy clay	Sandy clay loam	Sandy loam	Loamy sand		Pathogens/ Soil types	Interaction
Final nematode population (roots and suckers)							
Nematode alone	21360	28860	43680	49920	35955	706.8	813.6
Nematode + Fungus	17164	22560	25704	31200	24157		
Mean	9631	12855	17346	20280			
Final nematode population (soil)							
Nematode alone	18000	30000	50000	58000	39000	883.9	767.8
Nematode + Fungus	16000	25000	38000	44000	30750		
Mean	8500	13750	22000	25000			
Reproduction factor							
Nematode alone	7.87	11.77	18.73	21.58	14.99	0.14	0.28
Nematode + Fungus	6.63	9.51	12.74	15.04	10.98		
Mean	3.62	5.32	7.87	9.15			
[b]Root-knot index							
Nematode alone	0.87	1.25	1.75	2.15	1.50	0.05	0.09
Nematode + Fungus	0.75	1.00	1.40	1.62	1.19		
Mean	0.40	0.56	0.79	0.94			
[c]Roots and suckers infection							
Fungus alone	26.00	32.00	42.00	50.00	37.50	1.5	2.9
Nematode + Fungus	30.00	48.00	60.00	72.00	52.50		
Mean	14.00	20.00	25.50	30.50			

[a]Each value is an average of four replicates. [b]Root-knot index: 0 = 0%, 1 = 1-25%, 2 = 26-50%, 3 = 51-75%, 4 = 76-100%.
[c]Percent roots and suckers infection by *S. sclerotiorum*.

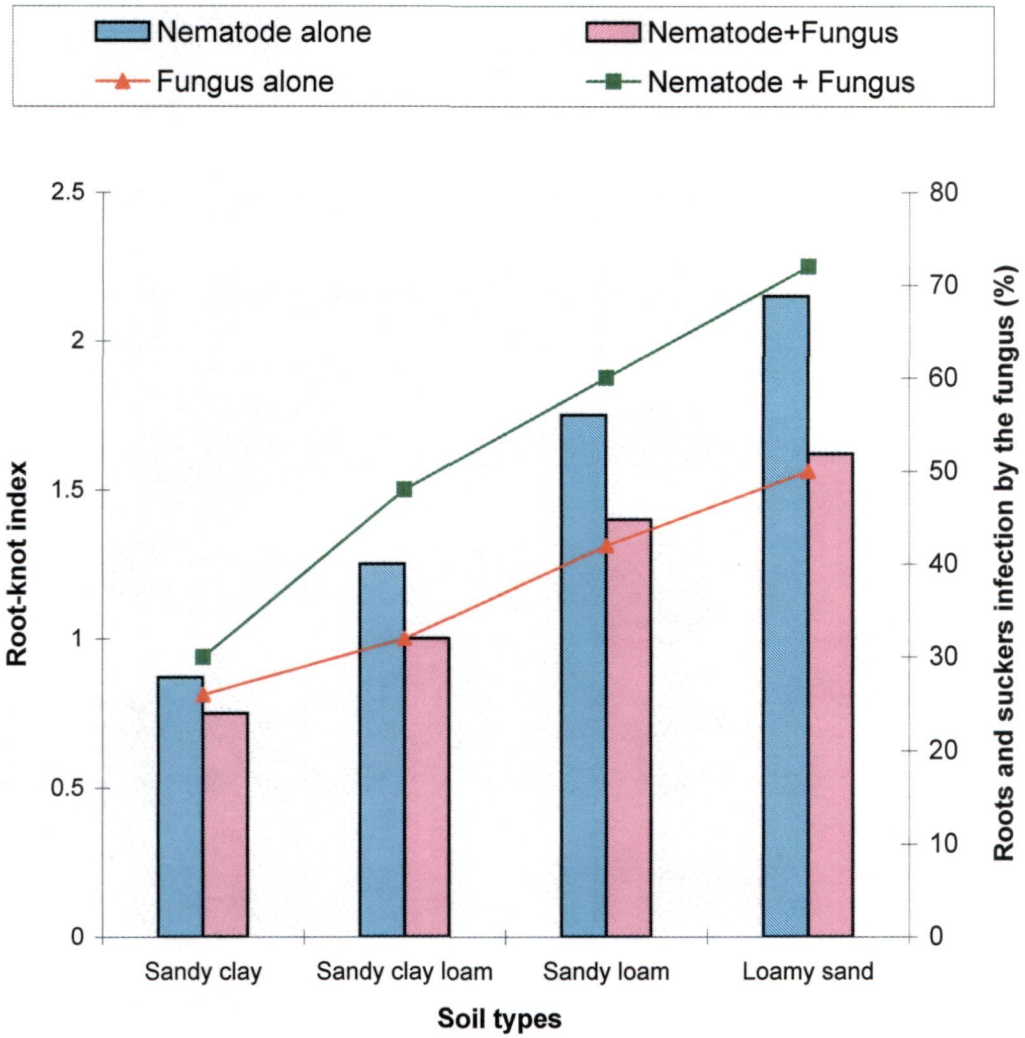


Fig.14: Effect of different soil types on disease development by *M. incognita* (5000 J2/5 kg soil) and *S. sclerotiorum* (3 g mycelium/5 kg soil) on *M. arvensis* cv. Gomti.

Root-knot index:- 0=0%, 1=1-25%, 2=26-50%, 3=51-75%, 4=76-100%.

reproduction rate, root-knot index and percent roots/suckers infection by fungus were observed in loamy sand followed by sandy loam, sandy clay loam and sandy clay soil, respectively. In loamy sand highest the reproduction factor (21.58) and root-knot index (2.15) were observed in plants inoculated with nematode alone, while maximum roots/suckers infection by fungus (72.00%) was observed in plants inoculated with fungus and nematode simultaneously.

Chlorophyll (a, b and total), total sugar and total phenol contents of the leaves were highest in plants grown in loamy sand soil followed by sandy loam, sandy clay loam and sandy clay, respectively, in uninoculated plants (Table 18). However, no trend was observed in inoculated plants. The highest reduction of plant chemicals was observed in loamy sand followed by sandy loam, sandy clay loam and sandy clay, respectively, as compared to uninoculated controls.

Reduction in chlorophyll a, chlorophyll b, total chlorophyll, total sugar and total phenol was observed highest (37.30, 36.99, 37.00, 35.29 and 39.20%, respectively) in plants grown in loamy sand soil and inoculated with nematode and fungus simultaneously.

Analyses of data indicated that significant ($P \leq 0.05$) reduction was observed in chlorophyll (a, b and total), total sugar and total phenol contents of inoculated plants as compared to uninoculated plants, irrespective of soil types. However, several non-significant differences were observed in chlorophyll a, chlorophyll b, total chlorophyll, total sugar and total phenol content of leaves of plants grown in different soil types as well as in plants inoculated with the two pathogens.

DISCUSSION

The effect of different soil types on various plant growth parameters of *M. arvensis* cv. Gomti was significant, both in the absence and presence of nematode and fungus. The highest reduction in plant growth and oil yield was observed in loamy sand soil followed by sandy loam, sandy clay loam and sandy clay, respectively as compared to corresponding uninoculated plants. The studies carried out by Windham and Barker (1986), Nakasono *et al.* (1990), Barker and

Table 18: Effect of different soil types on the biochemical changes in uninoculated, *Meloidogyne incognita* (5000 J2/5 kg soil) and/or *Sclerotinia sclerotiorum* (3 g mycelium/5 kg soil) inoculated plants of *Mentha arvensis* cv. Gomti.[a]

Parameters and treatments	Soil types				Mean	L.S.D. $_{0.05}$	
	Sandy clay	Sandy clay loam	Sandy loam	Loamy sand		Pathogens/ Soil types	Interaction
Chlorophyll content (mg/g fresh leaves)							
Chlorophyll a							
Uninoculated control	1.12	1.21	1.24	1.26	1.21	0.02	0.05
Nematode alone	1.07 (4.46)[b]	1.41 (5.78)	0.96 (22.58)	0.95 (24.60)	1.10		
Fungus alone	1.09 (2.68)	1.15 (4.96)	1.14 (8.06)	1.16 (8.73)	1.13		
Nematode + Fungus	1.04 (7.14)	1.06 (12.40)	0.80 (35.48)	0.79 (37.30)	0.92		
Mean	1.08	1.21	1.03	1.04			
Chlrophyll b							
Uninoculated control	0.60	0.67	0.70	0.73	0.67	0.02	0.04
Nematode alone	0.57 (5.00)	0.62(7.46)	0.53 (24.28)	0.54 (26.03)	0.56		
Fungus alone	0.58 (3.33)	0.64 (4.48)	0.64 (8.57)	0.66 (9.59)	0.63		
Nematode + Fungus	0.56 (6.67)	0.58 (13.43)	0.44 (37.14)	0.46 (36.99)	0.51		
Mean	0.58	0.63	0.58	0.60			
Total chlorophyll							
Uninoculated control	1.72	1.89	1.95	2.00	1.89	0.03	0.08
Nematode alone	1.65 (4.07)	1.77 (6.35)	1.50 (23.08)	1.50 (25.00)	1.60		
Fungus alone	1.68 (2.32)	1.80 (4.76)	1.79 (8.20)	1.82 (9.00)	1.77		
Nematode + Fungus	1.61 (6.39)	1.65 (12.70)	1.25 (35.90)	1.26 (37.00)	1.44		
Mean	1.66	1.78	1.62	1.64			
Total phenol (mg/g fresh leaves)							
Uninoculated control	10.17	10.90	11.55	12.50	11.28	0.16	0.32
Nematode alone	9.50 (6.59)	9.85 (9.63)	8.75 (24.24)	9.25 (26.80)	9.34		
Fungus alone	9.80 (3.64)	10.15 (6.88)	10.35 (10.38)	11.00 (12.00)	10.33		
Nematode + Fungus	9.15 (10.03)	9.10 (16.51)	7.40 (35.93)	7.60 (39.20)	8.31		
Mean	9.65	10.00	9.51	10.09			
Total sugar (mg/g fresh leaves)							
Uninoculated control	15.00	15.64	16.25	17.00	15.97	0.30	0.60
Nematode alone	14.00 (6.67)	14.00 (10.48)	12.85 (20.92)	13.00 (23.52)	13.46		
Fungus alone	14.50 (3.33)	14.85 (5.05)	14.85 (8.61)	15.15 (10.88)	14.84		
Nematode + Fungus	13.35 (11.00)	13.00 (16.88)	11.00 (32.31)	11.00 (35.29)	12.09		
Mean	14.21	14.37	13.74	14.04			

[a]Each value is an average of four replicates [b]Figures in parentheses are percent reduction over uninoculated control

Weeks (1991), Haseeb *et al.* (1999b) are also in agreement with the results obtained in present study indicating that the root-knot nematode causes greater damage to plants in lighter soil than in heavy soils. Similarly, Mazumder and Phookan (1996) and Srivastava and Kamthan (2002) observed that reduction in plant growth and yield due to fungi was more pronounced in coarse textured soil than fine textured soil.

The highest nematode population, reproduction rate, root-knot index was observed in loamy sand followed by sandy loam, sandy clay loam and sandy clay respectively. The results are in general agreement with findings of other workers (Windham and Barker, 1986; Nakasono *et al.,* 1989; Jordan *et al.*, 1989; Haseeb *et al.,* 1999b).

Similarly, the highest roots/suckers infection due to *S. sclerotiorum* was observed in plants grown in loamy sand soil followed by sandy loam, sandy clay loam and sandy clay soil, respectively. The results are in general agreement with the observations of Srivastava and Kamthan (2002) and Sarkar and Gupta (2002).

Plant growth and oil yield of *M. arvensis* cv. Gomti in different soil types were influnced by absence or presence of the nematode and the fungus. Generally, heavier soils have more water retention capacity, more adhesion and cohesion to retain nutrients and more organic matter than do light soils. Collectevely, these factors favour the plant growth and development (Thompson and Troeh, 1973), whereas, development of disease and multiplication of the nematode and the fungus was favoured by lighter soil, may be be due to better porosity in lighter soils, which may help in meeting the required oxygen demand of the pathogens and provide better space for multiplication (Norton, 1978; Sati and Sinha, 1999), and these factors may be responsibie for higher growth and oil yield of *M. arvensis* cv. Gomti in sandy clay than the other soil types.

The inoculation of *M. incognita* and *S. sclerotiorum* either alone or together reduces the chlorophyll, total sugar and total phenol content of the leaves of *M. arvensis* cv. Gomti, irrespective of soil types. Shukla and Haseeb (1999) also noticed the reduction in biochemical parameters of *M. spicata* cv.

MSS-5, inoculated with *P. thornei*, irrespective of soil types as compared to corresponding uninoculated control.

The result indicates the importance and complexity of soil types as a factor-affecting yield of *M. arvensis* cv. Gomti and multiplication of the pathogens. This information will be helpful for farmers in selecting a field for the cultivation of *M. arvensis* cv. Gomti.

Chapter 6

Management

8. Comparative efficacy of pesticides, neem seed powder and bio-control agents on root-knot disease development, nematode multiplication, growth and oil yield in *M. incognita* inoculated plants of *M. arvensis* cv. Gomti

Nematode management can be defined as a practice whereby phytopathogenic nematode populations are maintained at levels that do not inflict economic losses. Several methods have been in practice for the control of nematode, which include chemical and non-chemical.

Chemical methods include the use of fumigant and non-fumigant nematicides for the control of plant-parasitic nematodes (Taylor, 1975; Wright, 1981; Johnson, 1985; Johnson and Feldmesser, 1987). While, non-chemical methods are comprised of crop rotation, interculture, trap crops, green manuring, organic amendments and biological control (Linford *et al.*, 1938; Sayre, 1971; Norton, 1978; Jatala *et al.*, 1980; Fassuliotis, 1985; Rich and Hodge, 1993; Zukerman *et al.*, 1993).

REVIEW OF LITERATURE

Chemicals

Historically, one of the first major efforts to manage plant-parasitic nematodes was made in 1881 in Germany in sugarbeet industry, later on a breakthrough was achieved in 1943 and 1945 with the discoveries of the potential of DD and EDB, respectively as effective soil fumigants (Thorne, 1961). Non-fumigant chemicals were tested under field conditions nematicidal activity during the late 50's and early 60's, these materials are usually carbamates or organic phosphate and most, if not all of them were first tested for their insecticidal qualities and subsequently, their nematicidal activity was also investigated. Although numerous chemicals have been tested for nematicidal properties, very few have been developed and even fewer are currently used in managing plant-parasitic nematodes (Heald, 1987).

Davide and Zorilla (1980) reported that aldicarb, oxamyl, ethoprop and carbofuran significantly reduced the population of *M. incognita, Helicotylenchus* sp., *Rotylenchulus* sp. *Trichodorus* sp., *Pratylenchus* sp. and *Hoplolaimus* sp. infesting cabbage cv. KK. The nematicide treatments increased 25-87% yield of cabbage. Aldicarb gave the highest increase followed by oxamyl, carbofuran and ethoprop.

Rich and Green, Jr. (1981) reported that in the field tests, phenamiphos, aldicarb, carbofuran, BAS 263-04-1 and fensulfothion in 20 cm bands increased sunflower growth and reduced the populations of *M. javanica.* Phenamiphos at 3.0 kg a.i./ha was phytotoxic to the sunflower plants, but not at 1.2 kg a. i./ha.

Nordmeyer *et al.* (1982) evaluated the efficacy of ethoprop, carbofuran and aldicarb @ 6.7 kg a.i./ha in broadcasting against *M. javanica, M. incognita* and *M. arenaria* on flue-cured tobacco in the field, and found that aldicarb significantly increased yields of tobacco in presence of all the nematode species. In this study ethoprop, however, failed to increase yield of tobacco crop infected with *M. javanica,* whereas, carbofuran also did not gave significant increase in the crop yield. All the three nematicides increased plant height up to 70 days after planting with the exception of carbofuran in case of *M. javanica.* Gall index rating at final harvest was high and did not exhibit differences among the treatments.

Grandison (1983) reported that the bare-root immersion treatments with 0.1 and 1% a.i aqueous solutions of carbofuran, chlorine, isazophos, methomyl, oxamyl, parathion and phenamiphos effectively controlled the *M. hapla* infesting Kiwi fruit *(Actinidia chinensis).* Isazophos treatment at 0.1 and 1.0%, however, caused severe phytoxicity and death of some plants, the phytotoxic effect of parathion was moderate to severe whereas, it was moderate (1.0%) due to oxamyl.

Sanchez de Leon (1984) reported that the two applications at 45 and 95 days of intervals aldicarb @ 0.5 g/plant, carbofuran at 2.0 g/plant or marshal 250 EC at 25 ml/plant gave good control of *Meloidogyne* spp. and *P. coffeae* infesting coffee seedlings in nurseries.

Rodriguez-Kabana and King (1985) conducted a study continuously for five years to evaluate the relative efficacy of EDB (8.4 and 16.8 l/ha), ethoprop (2.2 and 4.4 kg a.i./ha) aldicarb, carbofuran, oxamyl and phenamiphos (1.1 and 2.2 kg a.i./ha) for the control of *M. incognita* on groundnut. All tested nematicides reduced the larval population of nematode in soil, at near harvest time and also increased the yields. Larval population in soil was negatively and linearly related to the amount of nematicides added. The most effective nematicides for suppressing larval populations were EDB and aldicarb, while carbofuran and ethoprop were the least effective.

Haseeb *et al.* (1988) studied the comparative efficacy of aldicarb (2.0 mg a.i./kg soil), carbofuran (1.5 mg a.i./kg soil) and carbendazim (1.0 mg a.i./kg soil) and neem and mahua cake (1.0 g N/kg soil) to control the *M. incognita* in *O. basilicum*. Various treatments showed an increase in the total plant length, fresh/dry weight and oil yield. However, neem cake provided the greatest improvement in plant growth, following the order of decreasing efficacy of aldicarb, carbendazim, mahua cake and carbofuran.

Badra and Adesiyan (1990) reported that aldicarb, carbofuran, isazophos or oxamyl at 2 or 4 kg a.i./ha applied to maize resulted a significant increase in yield. Cob yield was also increased by 24, 27, 28 and 31% with isazophos (4 kg a.i./ha), carbofuran (2 kg a.i./ha), aldicarb (2 kg a.i./ha) and isazophos (2 kg a.i./ha), respectively and grain yields by 43, 50, 65 and 84% with oxamyl (4 kg a.i./ha), aldicarb (2 kg a.i./ha), isazophos (4 kg a.i./ha) and isazophos (2 kg a.i./ha), respectively soil populations of *Pratylenchus safaensis, M. incognita, H. dihystera, H. galeatus* and *Criconemoides* sp. were suppressed by nematicides with isazophos and aldicarb being the most effective.

Babatola and Omotade (1991) evaluated the effect of carbofuran 3G (3 and 5 kg a.i./ha) and ethoprop 10G (2 and 4 kg a.i./ha) in a field trial during 1987 and 1988 for the control of *P. brachyurus* and *M. incognita* on cowpea. Nematode populations both in soil and roots of cowpea were reduced significantly by treatments with nematicides. Number of pods per plant, average number of grains per pod, weight of 100 grains and grain yields were

significantly improved by the treatments. Root gall indices were low in all the treatments but high in untreated plots.

Haseeb and Butool (1993) determined the effect of aldicarb, carbendazim, carbofuran, ethoprophos and ebufos @ 4 kg a.i./ha, and oil cakes of linseed, mustard and neem @ 1 g N/kg soil on *M. incognita* parasitizing *T. ammi*. In general, all the treatments were able to reduce the root-knot development and nematode population and increase the plant fresh and dry weight. Best results in terms of reduction in root-knot index and improvement in plant growth were achieved in plants treated with ethoprophos followed by carbofuran, neem cake, carbendazim, ebufos, mustard and linseed cakes respectively.

Besides preceding reports for the control of plant-parasitic nematodes particularly root-knot nematode by the nematicides, a large number of workers have also attempted to manage this nematode by the application of various nematicides (Rodriguez-Kabana, 1986; Rhoades and Forbes, 1986; Berbe'c and Dolna, 1990; Haseeb and Butool, 1991; Hartwig and Sikora, 1991; Sahoo and Das, 1995; Nanjegawda *et al.,* 1998; Pandey *et al.*, 1999; Javed and Ahmad, 1999; Khan and Rathi, 2001; Singh and Mittal, 2002).

Organic amendments

The application of organic amendments in the form of organic manures, dry crop residues, green manuring, meals and oil cakes, etc. for the control of plant-parasitic nematodes is an old practice which has received considerable attention in India and elsewhere (Sayre *et al.*, 1964; Singh and Sitaramaiah, 1973; Rodriguez-Kabana, 1986).

Amending the soil with commonly available parts and products of neem such as leaf, cakes, kernel, seed shell, etc., is one of the common methods used against plant-parasitic nematodes, especially in India. Leaves and cakes have commonly been used as soil amendments (Singh and Sitaramaiah, 1967, 1970, 1971, 1973; Alam *et al.,* 1980; Haseeb, 1983; Akhtar and Alam, 1989; Haseeb *et al.,* 1988, 1993; Haseeb and Butool, 1991; Alam, 1990; Shukla and Haseeb,

1996), and seed, seed kernel and seed coat have also been used (Majumder and Mishra, 1992; Dash and Padhi, 1998; Musabyimana and Saxena, 1999).

Khan *et al.* (1966, 1974) and Haseeb (1983) found that the water-soluble fractions of oil cakes suppress the population of nematodes in root and soil. They reported that nimbidin and thionemone alkaloids obtained from neem were deleterious to *T. brassicae, P. coffeae* and larvae of *M. incognita*. The population of *M. incognita* was considerably reduced by soil amendments with linseed, neem, groundnut and karanj oil cakes.

Singh and Sitaramaiah (1967) applied neem leaves @ 5-10% w/w in soil infested with *M. incognita* and got considerable reduction in the incidence of root-knot on tomato and okra. Singh and Sitaramaiah (1970, 1971, 1973) found that the various oil cakes when incorporated in to infested soil in the fields three weeks before planting of okra, tomato or potato, reduced the number of galls induced by *M. javanica.*

Lal and Hameed (1969) observed a significant reduction in the root- knot incidence due to *M. incognita* on tomato, okra and eggplant, incorporating chopped neem leaves in infested soil. The application of chopped leaves @ 5% to infested soil was also found to be effective against *R. reniformis* in eggplant (Lal *et al.,* 1977) and against other plant-parasitic nematodes viz. *Hop. indicus, Hel. indicus, T. brassicae* on chilli (Akhtar and Alam, 1989).

The significant reduction in root galling, egg mass production and population of *M. incognita,* as well as an increase in plant growth of a number of crops, by the application of organic amendments has been recorded by various workers (Majumder and Mishra, 1992; Poornima and Vadivelu, 1993; Haseeb *et al.,* 1988; Devi and Das, 1998; Dash and Padhi, 1998; Musabyimana and Saxena, 1999; Ravindra *et al.,* 2001).s

Bio-control agents

Nematicides form a small proportion (less than 2.5%) of total pesticide and herbicide usage (Wybou and Homeyer, 1984), and in general have not caused the major ecological problems that have developed with some other

chemicals. However, some compounds have been withdrawn from the market because of health hazards to production workers or because of their detection at unacceptable levels in ground water, whereas, other nematicides are also under threat. Unless more acceptable nematicides are developed, the strategies for nematode management will be forced to diversify in some other ways (Kerry, 1990). During the past two decades, the research on biological control of nematodes has been the subject of interest all over the world (Mankau, 1980a, b, 1981; Tribe, 1980; Jatala *et al.,* 1980; 1981; 1985; Morgan-Jones *et al.*, 1984; Rodriguez–Kabana *et al.*, 1984; Dube and Smart, 1987; Cabanillas and Barker, 1989; Walia *et al.,* 1991; Stephan *et al.*, 1998; Haseeb and Shukla, 2001a).

In an early study, Jatala *et al.* (1980) evaluated the efficiency of a bio-control agent, *Paecilomyces lilacinus* under field conditions and compared it with the chemical control. They investigated that the plants grown in plots inoculated with the fungus had significantly lower root gall index than those grown in plots in the presence of organic matter and nematicides. The efficacy of *P. lilacinus* against *Meloidogyne* species has also been studied on a variety of crops (Lay *et al.*, 1982; Godoy *et al.*, 1983; Noe and Sasser, 1984; Davide and Zorilla, 1986; Haseeb and Shukla, 2004).

Dube and Smart (1987) reported that the root-knot nematode was effectively controlled and thereby the yield of winter vetch also increased with the use of *P. lilacinus* and *Pasturia penetrans,* when applied together in comparison to alone.

Cabanillas *et al.* (1988) studied the histopathological changes in tomato roots inoculated with *P. lilacinus* and *M. incognita* race-1 and reported that the root galling and giant-cell formation were absent in tomato roots inoculated with nematode eggs infected with *P. lilacinus*. Few to no galls and no giant-cell formation were found in roots dipped in spore suspension of *P. lilacinus* and inoculated with *M. incognita*. Whereas, the formation of numerous large galls and giant cells was noticed in roots inoculated only with *M. incognita.* They further observed that *P. lilacinus* colonized the surface of epidermal cells as well as cortex cells.

Several reports are also available on the efficacy of *P. lilacinus* against plant-parasitic nematodes, especially root-knot nematodes (Khan and Esfahani, 1990; Saikia and Roy, 1994; Khan and Goswami, 1995; Anver and Alam, 1997; Nagesh *et al.*, 1997; Haseeb *et al.*, 1998, 2002; Holland *et al.*, 1999; Jayakumar *et al.*, 2002; Haseeb and Shukla, 2004).

In addition, attempts have also been made to explore the potential use of *Trichoderma* spp. to control the plant-parasitic nematodes by various workers (Windham *et al.*, 1989; Reddy *et al.*, 1996, Rao *et al.*, 1997, 1998; Sharon *et al.*, 2001; Pant and Pandey, 2002; Haseeb and Shukla, 2004).

In this regard, Windham *et al.* (1989) stated that the egg production of *M. arenaria* on maize was reduced in the soil treated with *T. harzianum* (T-12) and *T. koningii* (T-8).

Reddy *et al.* (1996) applied *T. harzianum* along with neem cake and noted reduction in the population of *T. semipenetrans*. Similarly, Rao *et al.* (1997) observed a significant increase in the plant growth and reduction in root galling and final population of *M. incognita* on tomato, when its seedlings were transplanted in soil incorporated with *T. harzianum* and amended with neem cake.

Sharon *et al.* (2001) evaluated the potential of *T. harzianum* against *M. javanica* on tomato cv. 144 and noted a reduction in the root galling and an increase in shoot fresh weight in nematode infected tomatoes treated with *T. harzianum*.

Pant and Pandey (2002) found that the application of neem cake and *T. harzianum* together reduced the incidence of *M. incognita* on chickpea followed by neem cake and *T. harzianum* alone respectively.

There have been a number of attempts to identify bacteria in bio-control programme of which most of the research work was centered on chitinolytic and rhizosphere inhabiting bacteria. Consequent upon the fluorescent *Pseudomonas* spp. have emerged as one of the promising group of bacteria for the bio-control of plant-parasitic nematodes (Oostendrop and Sikora, 1989).

Pseudomonas fluorescens strain A-59, T-58 and P-523 when applied as a seed or tuber treatment showed inhibition of early root penetration by *H. schachtii* and *G. pallida* in sugarbeet and potato under green house and field conditions respectively (Racke and Sikora, 1985; Oostendrop and Sikora, 1989; Hoffmann and Sikora, 1992).

The induction of galls due to *M. incognita* on tomato, cucumber and clover was also suppressed by *P. fluorescens* when applied as soil drench or root treatments under greenhouse conditions (Zavaleta-Meija and Van Gundy, 1982; Backer *et al.*, 1988; Shanthi and Sivakumar, 1995; Shanthi *et al.*, 1998).

Ramakrishnan *et al.* (1998) studied the effect of *P. fluorescens* against *Hirshmanniella gracilis* on rice and reported that *P. fluorescens* as seed treatment suppressed the nematode population and increased the crop yield by 13%. Similar observations have also reported by Seenivasan and Devrajan (2002) on rice with *P. fluorescens* against *H. gracilis*.

Aalten *et al.* (1998) reported that three strains of *P. fluorescens* and one type strain of *P. putida* (CF BP 2066) inhibited the invasion of *R. similis* and *Meloidogyne* spp. in the roots of banana, maize and tomato.

Jayakumar *et al.* (2002) reported that the culture filtrate of *P. fluorescens* strain PF-1 was found to be toxic to *R. reniformis,* however, the nematode mortality was increased with an increase in the exposure period and concentration of culture filtrates.

MATERIALS AND METHODS

8.1 Maintenance and production of bio-control agents

8.1.1 *T. harzianum*

Healthy sorghum seeds were soaked in 5 percent sucrose solution for 16 h. The seeds were strained and placed into 500 ml conical flasks to give 200 cm^3 of sorghum seeds/flask. Flasks with sorghum seeds were plugged with cotton and sterilized by autoclaving for 30 min at 1 kg/cm^2 pressure. The conical flasks containing sterilized sorghum seeds were inoculated with 1-cm-diameter PDA

discs punched from the periphery of actively growing 5-day-old culture of *T. harzianum.* Flasks were placed in an incubator at 27 ± 1°C, and the fungus was allowed to grow with periodic shaking of the flasks, so that the surface of all sorghum seeds were colonized and its colony forming units reached above 10^8 cfu/g culture.

8.1.2 *P. lilacinus*

P. lilacinus was multiplied on sorghum seeds in the manner as described in 8.1.1.

8.1.2 *P. fluorescens*

The culture tubes each containing 10 ml King's medium B (Broth) (King *et al.*, 1954) was autoclaved for 30 min at 1 kg/cm^2 pressure. When culture tubes were cooled, each tube was inoculated with the single colony of *P. fluorescens* strain Pf-1 from pure bacterial culture maintained on King's medium B agar. The culture tubes were placed in a BOD incubator for 48 h at 30 ± 1°C for the multiplication of *P. fluorescens.* For mass production, one-liter conical flasks containing 500 ml King's medium B (Broth) were autoclaved at the same pressure and time as mentioned above. When flasks were cooled, each flask was inoculated with 1.0 ml of *P. fluorescens* cultured broth. The flasks were kept at 30 ± 1°C in the BOD incubator for 120 h and were shaken twice a day. Inoculum culture was mixed with talc in the ratio of 1:4, and afterwards the amount of talc was adjusted so that the final cfu of *P. flucrescens* was maintained on 2x10^8 cfu/ml.

8.2 Application of treatments

To determine the effect of various management components, treatments and treatment combinations were applied into pots containing autoclaved 5 kg soil and farmyard manure (5:1 v/v) mixture were mixed thoroughly with various treatments and treatments combination, according to the following scheme.

S. No.	Treatments	Rate of application/ha	Rate of application/kg soil
1	Carbofuran (Furadan 3G)	3.0 kg a.i.	1.5 mg a.i.
2	Carbendazim (Bavistin 50%)	2.0 kg a.i.	1.0 mg a.i.
3	Neem seed powder	100 kg	50 mg
4	*Pseudomonas fluorescens* (10^8cfu/g)	100 kg	50 mg
5	*Paecilomyces lilacinus* (10^8cfu/g culture)	100 kg	50 mg
6	*Trichoderma harzianum* (10^8cfu/g culture)	100 kg	50 mg
7	Neem seed powder + Carbofuran	*	*
8	Neem seed powder + Carbendazim	*	*
9	Neem seed powder + *P. fluorescens*	*	*
10	Neem seed powder + *P.lilacinus*	*	*
11	Neem seed powder + *T. harzianum*	*	*
12	*P. lilacinus* + *P. fluorescens*	*	*
13	*P. fluorescens* + *T. harzianum*	*	*
14	*P. lilacinus* + *T. harzianum*	*	*
15	Carbofuran + Carbendazim	*	*

* In all combined treatments, rate of application was reduced to half of the standard rate.

The neem seed powder and bio-control agents were applied a week before the transplantation of sucker, while pesticides were applied a day before sucker transplanting. The treated pots were irrigated as needed to maintain good soil moisture. Four inoculated pots were left untreated and four pots were left uninoculated.

8.3 Transplanting and inoculation

In each pot, a single sucker was transplanted, and 5000 J2 of *M. incognita* were inoculated. The transplanting of sucker and inoculation of nematode was done in the same manner as described earlier in 2.3. For each treatment there were four replicates. The experiment was laidout as a completely randomized block design.

8.4 Recording of data

Recording of data regarding plant growth, oil yield and nematode population in soil and roots/suckers was done in the same manner as described in 2.4.1, 2.4.6, 1.5.1 and 1.5.2, respectively.

RESULTS

The application of various treatments and their combinations significantly increased the plant growth and oil yield of *M. arvensis* cv. Gomti as compared to untreated inoculated plants (Table 19; Fig.15). In comparison to untreated uninoculated plants, the least reduction in shoot height, shoot dry weight, roots/suckers dry weight and oil yield was observed in plants treated with neem seed powder + carbofuran, carbofuran alone and neem seed powder alone (Plate 16).

The influence of various treatments and treatment combinations on reproduction of *M. incognita* and root-knot index was inversely proportional to that on plant growth of *M. arvensis* cv. Gomti. The maximum control of nematode was achieved in neem seed powder + carbofuran treated plants (Rf = 1.65 and RKI = 0.25), whereas, the highest Rf (19.50) and RKI (1.87) were observed in untreated inoculated plants (Fig. 16).

Analyses of data indicated that maximum plant dry weight was recorded ($P \leq 0.01$) in plants treated with neem seed powder + carbofuran/carbofuran alone/neem seed powder alone followed by carbofuran + carbendazim/neem seed powder + *P. lilacinus*/neem seed powder + *T. harzianum*/neem seed powder + *P. fluorescens,* neem seed powder + carbendazim, *P. lilacinus* + *T. harzianum/P. fluorescens* + *P. lilacinus, P. fluorescens* + *T. harzianum, P. lilacinus* alone/*T. harzianum* alone/*P. fluorescens* alone and carbendazim alone respectively. The oil content of fresh herb was maximum ($P \leq 0.01$) in plants treated with neem seed powder + carbofuran/carbofuran alone/neem seed powder alone followed by neem seed powder + *P. lilacinus*/carbendazim + carbofuran/neem seed powder + *T. harzianum*; neem seed powder + carbendazim/neem seed powder + *P. fluorescens/P. lilacinus* + *T. harzianum/T.*

Table 19: Comparative efficacy of pesticides, neem seed powder and bio-control agents on root-knot disease development, nematode multiplication, growth and oil yield in *Meloidogyne incognita* (5000 J2/5 kg soil) inoculated plants of *Mentha arvensis* cv.Gomti.[a]

Treatments	Shoot height (cm)	Plant fresh weight (g)			Plant dry weight (g)			Final nematode population			Reproduction factor	[b]Root-knot index	Oil yield (ml/100 g fresh herb)
		Shoot	Roots & suckers	Total	Shoot	Roots & suckers	Total	Roots & suckers	Soil (5 kg)	Total			
Untreated uninoculated control	76.7	158.4	142.5	300.9	38.0	27.7	65.7	-	-	-	-	-	0.75
Untreated inoculated	64.0 (16.5)[c]	118.5 (25.2)	103.3 (27.5)	221.8	28.2 (25.8)	19.8 (28.5)	48.0	47518	50000	97518	19.50	1.87	0.58 (22.70)
Carbofuran	76.2 (0.6)	158.0 (0.2)	141.0 (1.0)	299.0	37.7 (0.8)	27.2 (1.8)	64.9	5640	6000	11640	2.33	0.37	0.72 (4.00)
Carbendazim	69.0 (10.0)	126.7 (20.0)	109.0 (23.5)	235.7	29.8 (21.6)	21.0 (24.2)	50.8	40330	46000	86330	17.27	1.62	0.62 (17.33)
Neem seed powder	75.8 (1.2)	158.0 (0.2)	140.8 (1.2)	298.8	37.6 (1.0)	27.0 (2.5)	64.6	5632	6000	11632	2.33	0.37	0.72 (4.00)
P. fluorescens	69.8 (9.0)	134.5 (15.1)	118.5 (16.8)	253.0	31.9 (16.0)	23.2 (16.2)	55.1	21330	23000	44330	8.87	0.95	0.64 (14.67)
P. lilacinus	71.5 (6.8)	138.0 (12.9)	122.5 (14.0)	260.5	32.7 (13.9)	23.6 (14.8)	56.3	18375	22000	40375	8.07	0.90	0.65 (13.33)
T. harzianum	70.3 (8.3)	137.1 (13.4)	120.0 (15.8)	257.1	32.6 (14.2)	23.0 (17.0)	55.6	19200	22000	41200	8.24	0.90	0.64 (14.67)
Neem seed powder + Carbofuran	76.2 (0.6)	158.2 (0.1)	142.0 (0.3)	300.2	37.8 (0.5)	27.6 (0.4)	65.4	4260	5000	9260	1.85	0.25	0.75 (0.0)
Neem seed powder + Cardendazim	74.0 (3.5)	147.2 (7.1)	129.7 (9.0)	276.9	35.0 (7.9)	25.0 (9.7)	60.0	15564	17000	32564	6.51	0.75	0.68 (9.30)
Neem seed powder + *P. fluorescens*	74.0 (3.5)	151.7 (4.9)	133.2 (6.5)	284.9	36.0 (5.3)	26.0 (6.1)	62.0	14652	16000	30652	6.13	0.70	0.68 (9.30)
Neem seed powder + *P. lilacinus*	75.0 (2.2)	152.5 (3.7)	135.5 (4.9)	288.0	36.4 (4.5)	26.2 (5.4)	62.6	12195	13000	25195	5.04	0.62	0.71 (4.00)
Neem Seed Powder + *T. harzianum*	74.6 (2.7)	152.0 (4.0)	135.0 (5.3)	287.0	36.1 (5.0)	26.0 (6.1)	62.1	13500	14000	27500	5.50	0.65	0.70 (6.67)
P. fluorescens + *P. lilacinus*	72.3 (5.7)	141.6 (10.6)	125.2 (12.1)	266.8	33.8 (11.0)	24.3 (12.6)	58.1	16276	20000	36276	7.25	0.87	0.66 (12.00)
P. fluorescens + *T. harzianum*	72.3 (5.7)	139.8 (11.7)	124.0 (13.0)	263.8	33.2 (12.6)	24.0 (13.3)	57.2	17360	20000	37360	7.47	0.87	0.66 (12.00)
P. lilacinus + *T. harzianum*	73.0 (4.8)	144.2 (8.9)	127.5 (10.5)	271.7	34.3 (9.7)	24.6 (11.2)	58.9	15300	18000	33300	6.66	0.75	0.60 (12.00)
Carbofuran + Carbendazim	75.5 (1.6)	155.2 (2.0)	137.5 (3.5)	292.7	36.9 (2.9)	26.5 (4.3)	63.4	11000	12000	22000	4.4	0.50	0.71 (4.00)
L.S.D. $_{0\,05}$	1.5	1.9	3.5	8.6	0.8	0.7	0.8	74.00	2658.0	3113.5	0.17	0.06	0.03
L.S.D. $_{0\,01}$	2.1	2.6	4.8	11.7	1.1	0.9	1.1	101.74	3587.7	4202.5	0.23	0.09	0.04

[a]Each value is an average of four replicates.
[b]Root-knot index : 0 = 0%, 1 = 1-25%, 2 = 26-50%, 3 = 51-75%, 4 = 76-100%.
[c]Figures in parentheses are percent reduction over untreated uninoculated control.

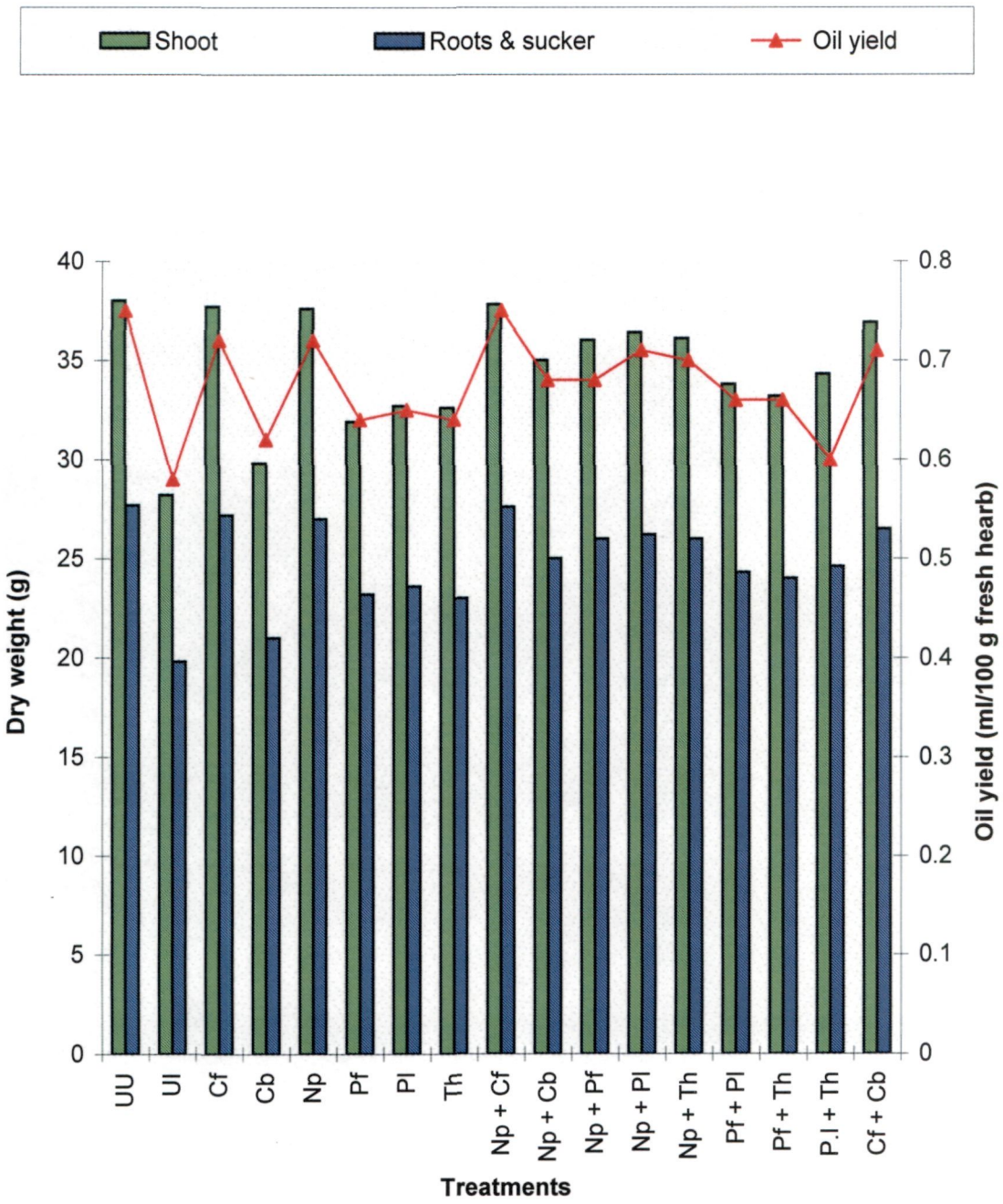


Fig.15: Comparative efficacy of various treatments on growth and oil yield of *M. incognita* (5000 J2/5 kg soil) inoculated plants of *M. arvensis* cv. Gomti.

UU= Uninoculated untreated
Cf= Carbofuran
Cb= Carbendazim
Np= Neem seed powder
Pf= *P. fluoroscens*
Pl= *P. lilacinus*
Th= *T. harzianum*

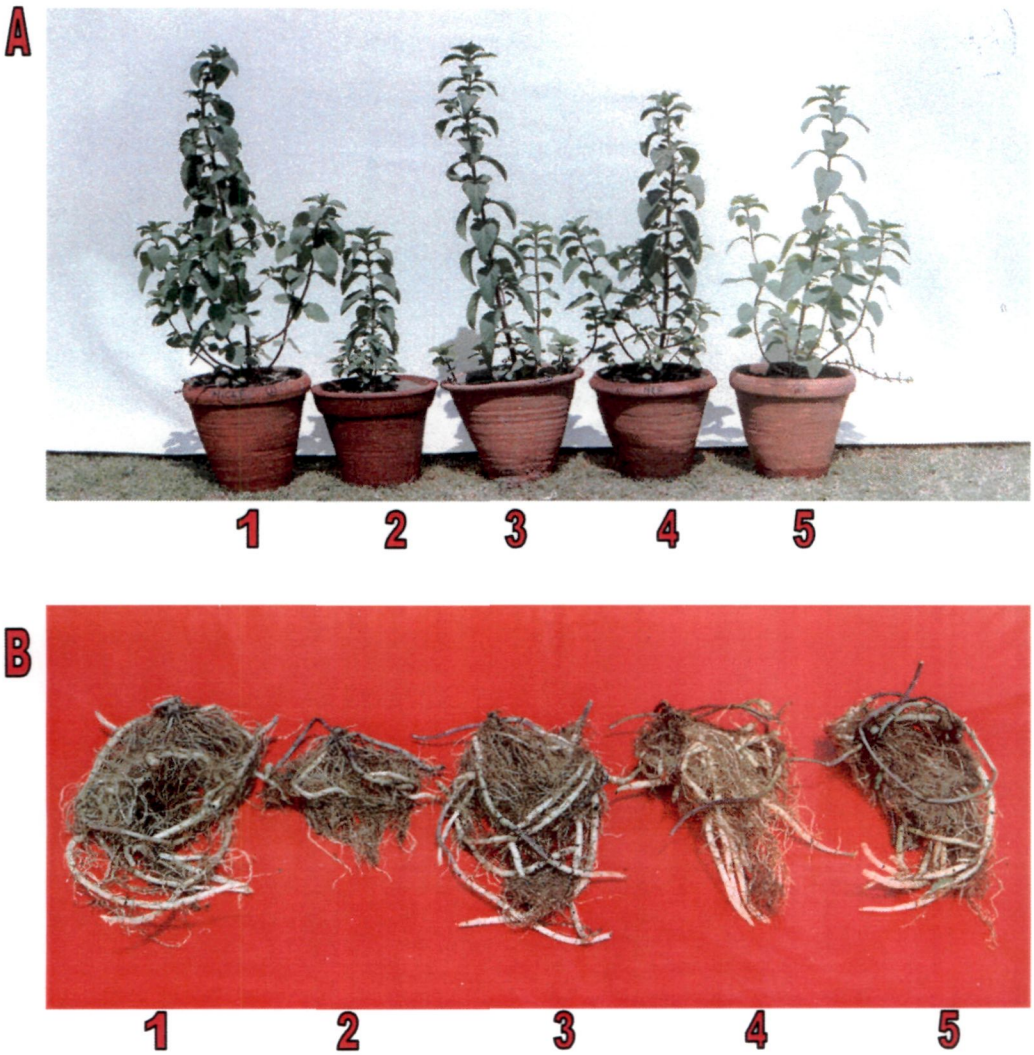


Plate 16: Effect of various treatments on aerial growth (A) and roots and suckers development (B) of *M. arvensis* cv. Gomti inoculated with *M. incognita* (5000 J2/pot). Showing the best three treatments.

1 = Uninoculated untreated

2 = Inoculated untreated

3 = Neem seed powder @ 250 mg/kg + carbofuran @ 0.75 mg a.i/kg

4 = Carbofuran alone @ 1.5 mg a.i/kg

5 = Neem seed powder alone @ 500 mg/kg

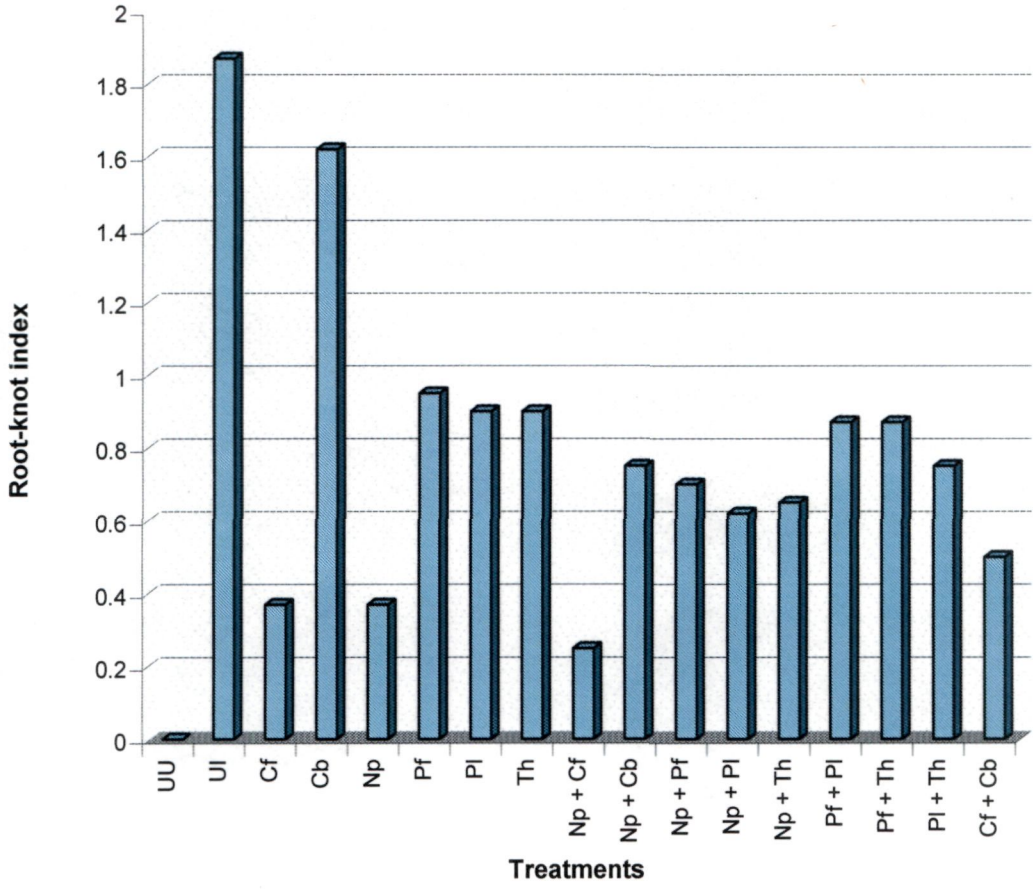


Fig.16: Comparative efficacy of various treatments on root-knot disease development in *M. incognita* (5000 J2/5 kg soil) inoculated plants of *M. arvensis* cv. Gomti.

Root-knot index:- 0 = 0%, 1 = 1-25%, 2 = 26-50%, 3 = 51-75%, 4 = 76-100%

UU = Uninoculated untreated
Cf = Carbofuran
Cb = Carbendazim
Np = Neem seed powder
Pl = *P. lilacinus*
Pf = *P. fluoroscens*
Th = *T. harzianum*

harzianum + *P. fluorescens/P. fluorescences* + *P. lilacinus; P. lilacinus* alone/*T. harzianum* alone *P. fluorescens* alone and carbendazim alone respectively. Similarly, analyses for reproduction of *M. incognita* indicated that the maximum reduction ($P \leq 0.01$) in reproduction rate was achieved by neem seed powder + carbofuran followed by carbofuran alone/neem seed powder alone, carbofuran + carbendazim, neem seed powder + *P. lilacinus*, neem seed powder + *T. harzianum*, neem seed powder + *P. fluorescens*, neem seed powder + carbendazim /*P. lilacinus* + *T. harzianum, P. fluorescens* + *P. lilacinus/P. fluorescens* + *T. harzianum, P. lilacinus* alone/*T. harzianum* alone, *P. lilacinus* alone and carbendazim alone, respectively.

DISCUSSION

The various treatments, viz. carbofuran, carbendazim, neem seed powder, *P. lilacinus*, *T. harzianum* and *P. fluorescens* used alone and in different combinations resulted an increase in the growth of *M. arvensis* cv. Gomti in comparison to untreated inoculated plants. Greatest improvement in plant growth and reduction in reproduction of *M. incognita* was achieved in plants treated with neem seed powder + carbofuran and in desending order carbofuran alone, neem seed powder alone, carbofuran + carbendazim, *P. lilacinus* + neem seed powder, *T. harzianum* + neem seed powder, *P. fluorescencs* + neem seed powder, neem seed powder + carbendazim, *P. lilacinus* + *T. harzianum*, *P. fluorescencs* + *P. lilacinus, P. fluorescencs* + *T. harzianum, P. lilacinus* alone, *T. harzianum* alone, *P. fluorescencs* alone and carbendazim alone, respectively. The effectiveness of these treatments, tested under study, has also been reported earlier (Singh and Sitaramaiah, 1973; Davide and Zorilla, 1980; Jatala *et al.*, 1980; Backer *et al.,* 1988; Windham *et al.,* 1989; Haseeb and Butool, 1993; Haseeb, 2003; Haseeb and Shukla, 2004).

It is evident from the results that carbofuran was effective in improving plant growth, oil yield and reducing nematode population. Wright (1981) holds the opinion that carbofuran a systemic and granular nematicide with non-fumigant action does not cause direct mortality but affects the nematode through

contact in soil. On the other hand it interferes with nematode feeding when nematicides get absorbed in plant system. Wright (1981) suggested that the nematicide inhibit acetyl-cholinesterase at the nerve synapse causing malfunctioning of the muscular and other organic systems in the nematode. Thus, the disruption of these systems may influence the nematode movement, behaviour and ultimately may alter the infection process of the parasitism, either by delaying or reducing the penetration (Sikora and Hertwig, 1991).

Amendment with neem seed powder applied alone or in combination was found to enhance the plant growth and oil yield and caused reduction in the nematode population. Several workers suggested that the nematode control might be due to the toxicity of decomposition products like ammonia, phenolics, etc. (Alam *et al.,* 1979; Singh *et al.*, 1980), or changed physical and chemical properties of soil or due to increased host resistance (Haseeb, 1983; Alam *et al.,* 1979). It was suggested that this induction of resistance was due to increased levels of phenolics in roots. Various explanations were given about the possible mechanisms of action of neem seeds/cakes. However, still more research is needed to find out the exact mechanism of action of neem seed powder for the control of the nematodes.

The results of present study clearly indicated the effectiveness of *P. lilacinus* in reducing plant damage caused by the nematode. Jatala *et al.* (1979) and Morgan and Rodriguez-Kabana (1984) also reported that reduction in the population of root-knot nematode results by fungal attack on J2 or death of females before egg laying, and reducing their fecundity, and if eggs are produced, they are colonized and destroyed by the fungus.

The possible mechanisms of action of *T. harzianum* against nematode are not as yet well defined. However, Saifullah and Thomas (1996) in an *in vitro* study demonstrated that *T. harzianum* penetrated the cysts and eggs of *Globodera rostochiensis* and caused the larval death. Khan and Saxena, (1997) found the metabolites of *T. viride* effective against nematodes. The root-knot nematode cuticle is mainly composed of proteins (Blaxster and Robertson,

1998); therefore, it may be assumed that the proteolytic activity of the antagonist lead to increased bio-control ability.

The present study revealed that *P. fluorescens* was also effective against *M. incognita* and improved plant growth. The probable mechanism responsible for the reduction of nematode population might be related to the ability of the bacteria to envelop or bind the root surface with carbohydrate lectin, thereby interfering with normal host recognition (Oostendorp and Sikora, 1989).

9. Efficacy of pesticides, neem seed powder and bio-control agents on disease development, growth and oil yield in *S. sclerotiorum* inoculated plants of *M. arvensis* cv. Gomti

In the modern age of intensive cropping, the disease problems become one of the important limiting factors for the crop production and therefore, the use of chemicals for the management of plant diseases has become not only important but forms an essential component of various inputs for increasing the crop productivity. The first important landmark in the management of plant diseases by chemicals is the discovery of Bordeaux mixture by Millardet in 1885.

REVIEW OF LITERATURE

Chemicals

A number of chemicals have been used to control Sclerotinia rots, which cause considerable losses in a variety of crops. The use of benomyl to control *S. sclerotiorum* has been found to be most effective on sunflower (Auger and Nome, 1970), beans (Natti, 1971), cabbage (Gabrielson *et al.*, 1973) and trellised tomato (Letham *et al.*, 1976).

In addition, the post-thinning spray application of Dicloran (0.1%) was found to be significantly effective and gave economical control of lettuce drop due to *S. minor* (Marcum *et al.*, 1977). Johnston and Springer (1978) evaluated the efficacy of Vinclozolin (BAS 352F) and found effective against the lettuce drop.

Singh and Gangopadhyay (1984) conducted a field experiment to control white rot of cauliflower caused by *S. sclerotiorum* and found that carbendazim @ 0.1% gave complete control of the disease and significantly increased the yield of the crop when sprayed at 10 days of intervals.

Singh and Saha (1989) tested *in vitro* the efficacy of carbendazim, kitazin, mancozeb, copper oxychloride, ziram, thiram and captan and concluded that all these chemicals were able to check the growth of *S. sclerotiorum*, the incitant of wilt and rot of knol-khol.

Singh *et al.* (1989) applied carbendazim fungicides (Agrozim, Bavistin, Derosal and Jkstein) or Brassicol (quintozene) @ 0.3% after the first cutting of *Trifolium alexandrinum* and found the suppression of disease incidence of *S. trifoliorum* and enhancement in the plant growth. The green fodder and seed yield were also increased by carbendazim and it was found to be more economical than quintozene.

Sharma and Munshi (1992) stated that carbendazim @ 2 kg a.i./ha was most effective followed by benomyl and captan, respectively against white mold of pea caused by *S. sclerotiorum*. Similarly for the management of Sclerotinia rot of Indian mustard, carbendazim and benomyl were found to be more effective than other fungicides when used as seed treatment (2.5 g/kg seed) or sprayed alone (2 kg a.i./ha) or in combinations with other fungicides (Singh *et al.*, 1994). On the other hand, Raj *et al.* (1995) treated seeds with carbendazim and chlorothalonil (@ 0.1%) and noted improvement in germination percentage and seedling vigour of sunflower inoculated with *R. oryzae* and *S. sclerotiorum*.

In vitro studies, Singh and Kapoor (1996) found that the carbendazim (25 µg a.i./ml) gave complete inhibition of mycelial growth of *S. sclerotiorum* on Richard's agar medium and no sclerotia production was noticed in carbendazim (50 µg, 37 µg a.i./ml), captan (250 µg a.i./ml) and metalaxyl + mancozeb (50 µg a.i./ml). These fungicides however, gave 100% inhibition of ascospore germination.

Kishore and Gupta (1997) observed that the translocation of carbendazim in sunflower was higher when seeds were treated at a dosage of 0.05% dissolved in acetone and carbon tetrachloride. Sclerotinia rot was effectively controlled (88.9 and 83.3% respectively) by these two treatments when compared to even higher dose (0.1%) of dry seed treatment, which gave only 66.7% disease control.

Shivpuri and Gupta (2001) reported that no sclerotial production by *S. sclerotiorum* was observed in the PDA plates amended with carbendazim, thiophanate methyl and phenylpyrrole. Among various plant extracts used for the control of the fungus, *Azadirachta indica, Datura stramonium, Ocimum sanctum, Vinca rosea, Withania somnifera, Polyalthia longifolia* and *Tagetes erecta* were observed to be more fungitoxic than others.

Sugha (2001), while trying integration of pre-sowing application of carbendazim granules @ 10 kg/ha and seed treatment with combination of carbendazim + thiram 1:1 @ 2.5 g/kg seed followed with three sprays of carbendazim 50 WP @ 0.1% at fortnightly intervals starting with the initiation of flowering found excellent (97.7%) control of white rot of pea and five fold increase in yield over check plots than any other treatments alone.

Kumawat and Jain (2003) tested the efficacy of various fungicides against stem rot of chickpea incited by *S. sclerotiorum* and reported that among all the fungicides tested against seed borne mycelia using blotter technique, benlate was found to be the most effective in checking growth of the fungus followed by topsin-M, carbendazim 25 WP, captan, thiram and zetron. Whereas, in seed treatments with benomyl @ 0.2%, carbendazim 50 WP @ 0.2%, thiophanate methyl @ 0.2% and carbendazim 25 WP @ 0.4% were found to be highly effective in checking pre-emergence seed rotting/seedling mortality. In foliar sprays, Benomyl (0.1%) was also noted to be the most effective followed by carbendazim (0.1%) and thiophanate methyl (0.1%), whereas, amongst non-systemic fungicides chlorothalonil (0.2%) was proved to be most effective.

In the last two decades, the interest has also increased in the potential and possible use of new bioactive products and natural pesticides, which cause less ecological damage (Patrick and Taussoun, 1965). The derivatives of neem such as oils, extracts and cakes have been found to be effective against fungal pathogens *in vitro* and *in vivo*. An early report of Singh *et al.* (1980) suggested the possible role of oil and extracts of *A. indica* in the control of soil-borne pathogens of *Cicer arietinum*. They reported that the growth of *F. oxysporum* f. sp. *ciceri, R. solani, S. rolfsii* and *S. sclerotiorum* was inhibited in liquid medium by extracts of leaf, trunk, bark, fruit pulp and oil of neem.

Singh and Singh (1981) concluded that the extracts of neem and neem oils (100-5000 ppm) adversely affected the germination and germ tube growth of conidia of *E. polygoni,* causing powdery mildew in field peas.

Vir and Sharma (1985) stated that adding of neem oil @ 2.5 and 5% in the agar medium may inhibit the radial growth of *F. moniliforme* by 57.8 and 66.7% respectively. However, the neem oil inhibited 77.8% growth of *A. niger*, 83.3 to 88.9% of *Drachslera rostratum* and 61.1 to 75.6% of *M. phaseolina.*

Sharma and Sharma (1986) noticed that the incidence of stalk rot of cauliflower caused by *S. sclerotiorum* was reduced by soil amendments of sunflower and rapeseed cakes. Whereas, Gupta *et al.* (1986) tried sunflower cake mustard cake, and other materials like gypsum and bean straw and found effective control of the disease incidence.

The control of phytopathogenic fungi by incorporating organic amendments such as cakes, oils, leaves, plant extracts have also been reported by various workers (Haseeb, 1983; Singh and Vyas, 1984; Hasabins and D'souza, 1987; Singh and Prasad, 1993; Barros *et al.,* 1995; Thakur *et al.*, 1995; Kazmi *et al.,* 1995; Shivpuri and Gupta, 2001; Singh and Majumdar, 2001; Haseeb and Shukla, 2002; Haseeb and Shukla, 2004).

Bio-control agents

A natural control of fungal pathogens can be obtained by the antagonistic activities of other microorganisms, which include actinomycetes, bacteria and fungi. Most of the work on the control of *S. sclerotiorum* and its allied species has been worked out with, fungal antagonists, such as *Coniothyrium minitans*, *Sporidesmium sclerotivorum*, *Trichoderma* spp., *Gliocladium roseum*, *Trichothecium roseum*, *Fusarium* spp., *Mucor* spp., *Alternaria* spp., *Epicoccum* spp. and *Penicillium* spp. (Tribe, 1957; Rai and Saxena, 1975; Merriman, 1976; Willetts and Wong, 1980). However, the antagonistic effects of bacteria and actinomycetes on *Sclerotinia* spp. have not been well studied.

Tu (1980) reported that the *G. virens* was able to inhibit the formation of sclerotia by *S. sclerotiorum*. *G. virens* parasitized both mycelia and sclerotia of the fungus and profusely sporulated on the sclerotia.

Gupta and Agarwala (1988) found that all the fungi isolated from the rhizosphere of cauliflower plants inhibited the growth of *S. sclerotiorum*, however, maximum antagonism was exhibited by *T. viride* followed by *A. terreus*, *Rhizopus arrhizus* and *F. solani*.

Dohroo *et al.* (1990) studied the antagonistic activity of some fungi against the *S. sclerotiorum* and they reported that the maximum inhibition of *S. sclerotiorum* was obtained with *T. viride* followed by *T. harzianum* and *G. virens*.

Several workers have also reported the antagonistic potential of *Trichoderma* sp. against *S. sclerotiorum* (Sharma and Singh, 1990; Knudsen *et al.*, 1991; Sharma *et al.*, 1992; Sharma *et al.*, 1996; Sharma and Basondrai, 1997; Sharma *et al.*, 1999; Singh *et al.*, 2003). Some strains of *T. harzianum* have also been reported to be antagonists of mycelium or sclerotia of *B. cinerea* (Zimand *et al.*, 1991, 1994, 1996; Elad *et al.*, 1993).

Fluorescent pseudomonads commonly found in soil, were effective colonizers of the rhizosphere of many crop plants and have been found to inhibit

the growth of a number of phytopathogenic fungi (Weller, 1988; Gutterson, 1990).

Kloepper *et al.* (1980) demonstrated that the many strains of fluorescent pseudomonads, which are termed as plant growth promoting rhizobacteria (PGPR), exert their plant growth promoting activity and act as biological control agent by producing extra cellular siderophores (microbial iron transport agents) which efficiently complex environmental iron, making it less available or unavailable to pathogens.

Howell and Stipanovic (1979) reported that the treatment of cotton seed with *P. fluorescens* or by the antibiotic Pyrrolnitrin isolated from *P. fluorescens* before planting in soil infested with *R. solani* increased the seedling survival from 30 (untreated) to 79% and from 13 to 70% respectively.

Howell and Stipanovic (1980) reported that in an *in vitro* study the strain Pf-5 of *P. fluorescens* was found antagonist to *P. ultimum.* An antibiotic, pyoluteorin isolated from Pf-5 strain cultures of *P. fluorescens* was found to be inhibitory to *P. ultimum.* Treatment of cottonseed with pyoluteorin or with *P. fluorescens* at the time of planting in *P. ultimum* infested soil, the seedling survival from 33 to 65% and from 28 to 71%, respectively.

The application of *P. fluorescens* as a bio-control agent has been advocated by several workers against the plant diseases caused by fungal plant pathogens such as *R. solani* (Howell and Stiponovic, 1979; Mew and Rosales, 1986; Savithiry and Gnanamanickan, 1989; Devi *et al.,* 1989; Thara and Gnanamanikam, 1994), *Pythium* spp. (Loper, 1988; Kaiser *et al.*, 1989), *F. oxysporum* (Park *et al.*, 1988; Vidhyasekaran and Muthamilan, 1995; Rangeshwaran *et al.*, 2001) and *Gaeumannomyces graminis* var. *tritici* (Weller, 1983; Weller and Cook, 1983; Thomashow and Weller, 1988; Weller *et al.*, 1988; Capper and Higgins, 1993).

MATERIALS AND METHODS

9.1 Application of treatments

The preparation of pots and application of treatments were done in the same manner as described in 8.1.

9.2 Transplanting and inoculation

A single sucker was transplanted into each treated pot and 3 g mycelium/5 kg soil of *S. sclerotiorum* was inoculated. The procedure for transplanting and inoculation was same as described earlier in 4.2. There were four replicates for each treatment. Four inoculated pots were left untreated and four pots were left uninoculated. The experiment was laidout as a completely randomized block design.

9.3 Recording of data

Recording of data regarding plant growth, oil yield, percent roots/suckers infection was done in the same manner as described earlier in 2.4.1, 2.4.6 and 1.3.2, respectively.

RESULTS

All the treatments significantly increased the plant growth and oil yield of *M. arvensis* cv. Gomti as compared to untreated inoculated plants (Table 20; Fig. 17). The least reduction in shoot height, shoot dry weight, roots/suckers dry weight and oil yield was observed in plants treated with neem seed powder + carbendazim, carbendazim alone and neem seed powder alone (Plate 17).

The extent of roots/suckers infection by *S. sclerotiorum* was decreased by the application of treatments and treatment combinations as compared to the roots/suckers infection due to fungus in untreated inoculated plants. The maximum control of roots/suckers infection by fungus was achieved by neem seed powder + carbendazim and carbendazim alone treatments. The effect of various treatments and treatment combinations on root infection by the fungus was observed inversely proportional to that on plant growth (Table 20; Fig. 18).

Plant dry weight data indicate that neem seed powder + carbendazim, carbendazim alone and neem seed powder alone were the best treatments ($P \leq$

Table 20: Comparative efficacy of pesticides, neem seed powder and bio-control agents on disease development, growth and oil yield in *Sclerotinia sclerotiorum* (3 g mycelium/5 kg soil) inoculated plants of *Mentha arvensis* cv. Gomti.[a]

Treatments	Shoot height (cm)	Plant fresh weight (g)			Plant dry weight (g)			[b]Roots & suckers infection	Oil yield (ml/100 g fresh herb)
		Shoot	Roots & suckers	Total	Shoot	Roots & sucker	Total		
Untreated uninoculated	73.3	144.0	132.0	276.0	34.4	25.5	59.9	-	0.72
Untreated inoculated	66.0 (9.9)[c]	115.0 (20.1)	104.0 (21.2)	219.0	27.2 (20.9)	20.0 (21.6)	47.2	45.0	0.66 (8.3)
Carbofuran	68.0 (7.2)	120.0 (16.7)	108.2 (18.0)	228.2	28.5 (17.1)	20.6 (19.2)	49.1	38.0	0.67 (6.9)
Carbendazim	72.0 (1.8)	140.5 (2.4)	129.2 (2.1)	269.7	33.4 (2.9)	25.0 (2.0)	58.4	12.0	0.70 (2.8)
Neem seed powder	72.0 (1.8)	142.9 (1.0)	128.2 (2.9)	270.0	33.8 (1.7)	24.8 (2.7)	58.8	8.0	0.72 (0.0)
P. fluorescens	68.0 (7.2)	130.2 (9.6)	115.0 (12.9)	245.2	30.8 (10.5)	22.0 (13.7)	52.8	34.0	0.67 (6.9)
P. lilacinus	67.7 (7.6)	125.0 (13.2)	111.0 (15.9)	236.0	29.7 (13.7)	21.8 (14.5)	51.5	38.0	0.67 (6.9)
T. harzianum	68.3 (6.8)	131.8 (8.5)	116.2 (12.0)	248.0	31.6 (8.1)	22.2 (12.9)	53.8	35.0	0.68 (5.5)
Neem seed powder + Carbofuran	70.2 (4.2)	136.2 (5.4)	121.5 (7.9)	257.7	32.3 (6.1)	23.4 (8.2)	55.7	25.0	0.68 (5.5)
Neem seed powder + Carbendazim	72.6 (0.9)	142.2 (1.2)	130.8 (0.9)	271.4	33.8 (1.7)	25.2 (1.2)	59.0	8.0	0.72 (0.0)
Neem seed powder + *P. fluorescens*	71.0 (3.1)	137.6 (4.4)	124.4 (5.7)	262.0	32.5 (5.5)	24.0 (5.9)	56.5	20.0	0.69 (4.2)
Neem seed powder + *P. lilacinus*	70.7 (3.5)	137.0 (4.9)	122.8 (7.0)	259.8	32.6 (5.2)	23.7 (7.0)	56.3	22.0	0.68 (5.5)
Neem seed powder + *T. harzianum*	71.5 (2.4)	138.2 (4.0)	125.3 (5.1)	263.5	33.0 (4.1)	24.0 (5.9)	57.0	20.0	0.69 (4.2)
P. fluorescens + *P. lilacinus*	68.5 (6.5)	133.5 (7.3)	117.7 (10.8)	251.2	31.5 (8.4)	22.5 (11.8)	54.0	30.0	0.68 (5.5)
P. fluorescens + *T. harzianum*	69.6 (5.0)	135.0 (6.2)	119.6 (9.3)	254.6	32.0 (7.0)	23.0 (9.8)	55.0	28.0	0.68 (5.5)
P. lilacinus + *T. harzianum*	69.5 (5.2)	134.2 (6.8)	118.7 (10.1)	252.9	31.8 (7.5)	22.8 (10.6)	54.6	28.0	0.68 (5.5)
Carbofuran + Carbendazim	71.7 (2.2)	139.0 (3.5)	126.2 (4.4)	265.2	33.0 (4.1)	24.2 (5.1)	57.2	12.0	0.70 (2.8)
L.S.D. $_{0\,05}$	4.3	6.4	6.6	10.1	0.5	0.4	0.5	3.9	0.02
L.S.D. $_{0\,01}$	5.8	8.6	9.0	13.7	0.7	0.5	0.7	5.2	0.03

[a]Each value is an average of four replicates.
[b]Percent roots and suckers infection by *S. sclerotiorum*.
[c]Figures in parentheses are percent reduction over untreated uninoculated control.

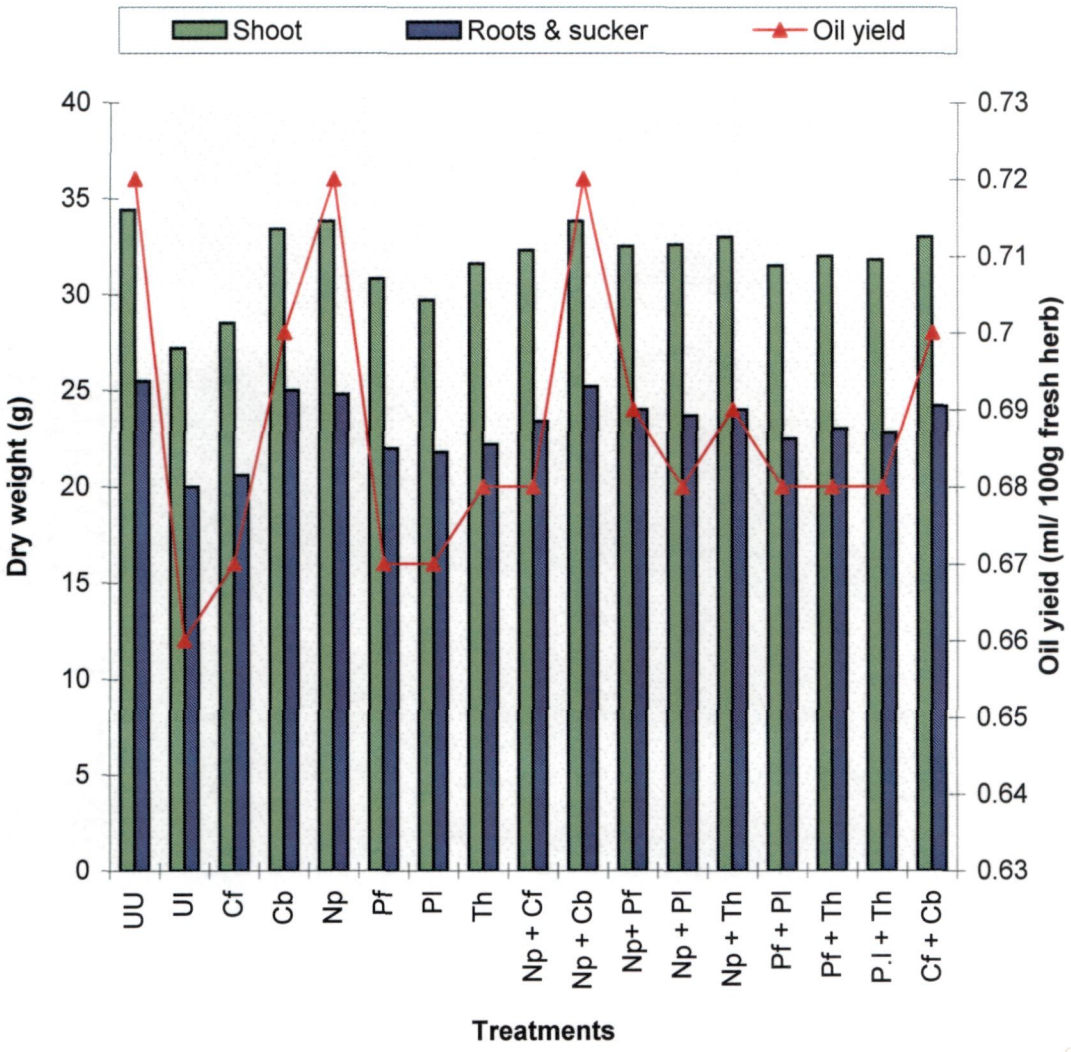


Fig. 17: Comparative efficacy of various treatments on growth and oil yield of *S. sclerotiorum* (3 g mycelium/5 kg soil) inoculated plants of *M. arvensis* cv. Gomti.

UU = Uninoculated untreated Cf = Carbofuran Cb = Carbendazim
Np = Neem seed powder PI = *P. lilacinus* Pf = *P. fluoroscens*
Th = *T. harzianum*

Plate 17: Effect of various treatments on aerial growth (A) and roots and suckers development (B) of *M. arvensis* cv. Gomti inoculated *S. sclerotiorum* (3 g mycelium/pot). Showing the best three treatments.

1 = Uninoculated untreated
2 = Inoculated untreated
3 = Neem seed powder @ 250 mg/kg + carbendazim @ 0.5 mg a.i./kg
4 = Carbendazim alone @ 1 mg a.i./kg
5 = Neem seed powder alone @ 500 mg/kg

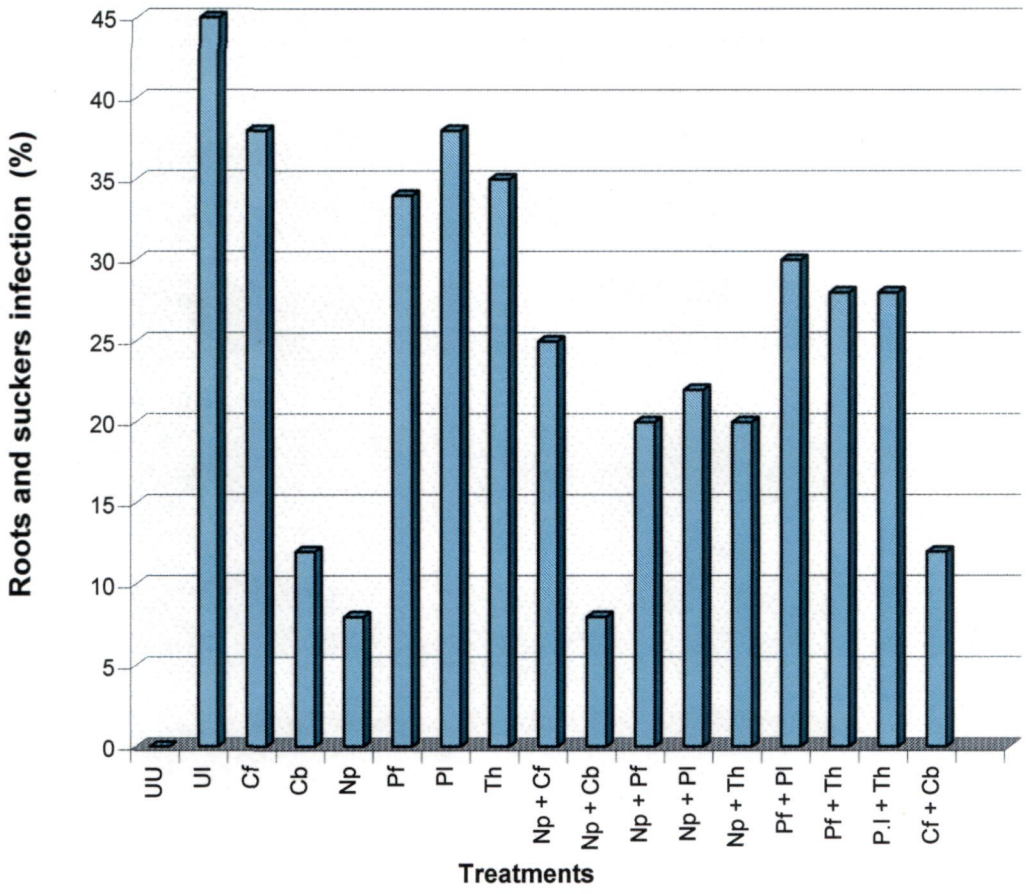


Fig. 18: Comparative efficacy of various treatments on disease development in *S. sclerotiorum* (3 g mycelium/5 kg soil) inoculated plants of *M. arvensis* cv. Gomti.

UU = Uninoculated untreated Cf = Carbofuran Cb = Carbendazim
Np = Neem seed powder PI = *P. lilacinus* Pf = *P. fluoroscens*
Th = *T. harzianum*

0.01) in improving plant weight followed by carbofuran + carbendazim/neem seed powder + *T. harzianum*/neem seed powder + *P. fluorescens*/neem seed powder + *P. lilacinus,* neem seed powder + carbofuran, *P. fluorescens* + *T. harzianum/P. lilacinus* + *T. harzianum, P. fluorescens* + *P. lilacinus/T. harzianum* alone, *P. fluorescens* alone, *P. lilacinus* alone and carbofuran alone, respectively. Similarly, neem seed powder + carbendazim/carbendazim alone/neem seed powder alone/carbofuran + carbendazim were the best treatments ($P \leq 0.01$) in improving oil yield followed by neem seed powder + *P. fluorescens*/neem seed powder + *T. harzianum/T. harzianum* alone/neem seed powder + carbofuran/neem seed powder + *P. lilacinus/P. fluorescens* + *P. lilacinus/T. harzianum* + *P. lilacinus/P. fluorescens* + *T. harzianum,* carbofuran alone/*P. fluorescens* alone/*P. lilacinus* alone, respectively. Analyses for percent roots/suckers infection by fungus indicates that neem seed powder + carbendazim/carbendazim alone/neem seed powder alone/carbofuran + carbendazim were the best treatments ($P \leq 0.01$) for decreasing root infection, followed by neem seed powder + *T. harzianum*/neem seed powder + *P. fluorescens*/neem seed powder + *P. lilacinus*/neem seed powder + carbofuran, *P. fluorescens* + *T. harzianum/P. lilacinus* + *T. harzianum, P. fluorescens* + *P. lilacinus/T. harzianum* alone, *P. fluorescens* alone, *P. lilacinus* alone and carbofuran alone, respectively.

DISCUSSION

The results of comparative efficacy of pesticides, neem seed powder and bio-control agents against *S. sclerotiorum* revealed that all the treatments were able to boost the plant growth and provide the satisfactory reduction in disease incidence (Table 20). The greatest plant growth and lowest root infection was observed in the plants treated with neem seed powder + carbendazim and in descending order, carbendazim alone, neem seed powder alone, carbofuran + carbendazim, neem seed powder + *T. harzianum*, neem seed powder + *P. fluorescens*, neem seed powder + *P. lilacinus*, neem seed powder + carbofuran, *P. fluoresens* + *T. harzianum, P. lilacinus* + *T. harzianum, P. fluorescens* + *P. lilacinus, T. harzianum* alone, *P. fluorescens* alone, *P. lilacinus* alone and

carbofuran alone, respectively. The present findings are similar to the results obtained by previous workers on various crops (Singh *et al.*, 1980; Dohroo *et al.*, 1990; Knudsen *et al.*, 1991; Singh and Kapoor, 1996; Kumawat and Jain, 2002; Singh *et al.*, 2003).

The fungus suppressing effect of carbendazim could be attributed to the fact that when this fungicide comes in contact with the plant, it gets converted at the plant surface to methyl benzimidazole carbamate and this compound interferes with nuclear division and biosynthesis of new cell material required for growth and maintenance of the fungi (Nene and Thapliyal, 2000).

Various workers have indicated that the neem tree (*Azadirachta indica* Juss.) contains several active principals, which are responsible for fungicidal activity (Narayanan and Ayer, 1967; Barak and Chakraborty, 1969). It might be possible that any of these chemicals present in neem seed powder inhibited the fungal growth or the sclerotia formation. Also Marukawa *et al.* (1975) noted that the P-amino benzoic acid present in neem exudates inhibited the sclerotia formation of *Sclerotinia liberitiana.*

The perusal of literature showed that *T. harzianum* has not been investigated extensively as a bio-control agent of *S. sclerotiorum.* Elad (1995) suggested various mechanisims for the bio-control activity of *Trichoderma* spp. against phytopathogenic fungi, such as antibiosis, competition, mycoparasitism and enzymatic hydrolysis, whereas, various workers reported *T. harzianum* as antagonist of mycelium or sclerotia of numerous plant pathogens (Lewis and Papavizas, 1987; Elad *et al.*, 1993; Vavrac *et al.*, 1997). The ability of these mycoparasitic fungi to attack sclerotia which results in reducing pathogen inoculum or source of inoculum in the soil seems to be a potential means of bio-control.

The findings of present studies showed that *P. fluorescens* was able to improve the plant growth and reduces root infection due to fungus. Kloepper *et al.* (1980) demonstrated that the PGPR exert their plant growth promoting activity and afford biological control by producing extra cellular siderophores

(microbial iron transport agents) which efficiently complex environmental iron and make it less available or unavailable to pathogens. Also, other secondary metabolites like antibiotics (Thomashow and Weller, 1990) and cyanide (Voisard *et al.,* 1989) have also been reported to be associated with the antagonism exerted by the pseudomonands.

10. Management of *M. incognita* and *S. sclerotiorum* disease complex of *M. arvensis* cv. Gomti

It is now well documented that the diseases of complex etiology involve nematodes and soil borne pathogens, which cause significant losses in agricultural crops. Therefore, the disease management strategies are being modified to specifically reduce the amount of damage caused by the interactions of nematode-fungal pathogens. In most of the cases, control of the nematode component of an interaction is fundamental objective in controlling the disease complexes (Nasbaum and Todd, 1970; Haseeb, 1983; Lanjewar and Shukla, 1985; Mangat and Bhatti, 1986; Parveen and Ghaffar, 1998; Haseeb *et al.,* 2001b; Haseeb and Shukla, 2001, 2002, 2004; Haseeb, 2003).

REVIEW OF LITERATURE

Parveen *et al.* (1993) studied the management of *M. javanica-F. oxysporum* disease complex of tomato and okra by fungal bio-agents and reported that *T. harzianum, T. koningii* and *G. virens* were found to be effective to control the *M. javanica* population on tomato and okra in the soil naturally infested with *F. oxysporum*. Whereas, *Bradyrhizobium japonicum, P. lilacinus* and carbofuran were able to control the gall formation on tomato and okra plants grown both in natural soil and in soil artificially infested with *F. oxysporum*.

Shahzad and Ghaffar (1996) managed *F. oxysporum* and *M. incognita* infecting mung bean by benomyl, captan, vitavax, mancozeb, tolclofos-methyl and carbofuran. They reported that carbofuran @ 2 kg a.i./ha significantly reduced the root-knot index but there was no effect on root colonization index by the fungus (RCI), whereas, soil drench with benomyl and mancozeb @ 5 kg

a.i./ha significantly reduced RCI with no effect on *M. incognita* infection. The combined application of carbofuran and fungicides showed significant reduction both in root-knot development and root colonization index.

Nagesh *et al.* (1997) evaluated *P. lilacinus, T. harzianum, T. viride* and neem cake alone and in combinations to manage the wilt complex of *Gladiolus* caused by *M. incognita* and *Fusarium*. They reported that both *T. harzianum* and *T. viride* controlled the wilt in the presence of *M. incognita* up to six weeks after emergence. The combined application of *P. lilacinus* + *T. harzianum* + neem cake and *P. lilacinus* + *T. viride* + neem cake was able to control both *M. incognita* and *Fusarium* till the harvest of flower spikes.

Latha *et al.* (2000) conducted a field trial to study the effect of chemicals and antagonists on root-rot disease complex of black gram caused by *M. phaseolina* and *H. cajani*. They reported that the combination of carbendazim as seed treatment (1.0 g/kg of seed) with carbofuran as soil application (3.3 kg a.i./ha) was found to be the most effective in reducing the root rot incidence, nematode population and increasing the pod yield. Among the bio-control agents combination of *P. lilacinus* with *T. viride* and *P. fluorescens* as seed treatment resulted in lesser root rot incidence, nematode population and more pod yield than other treatments.

Singh and Goswami (2001) studied the management of disease complex caused by *M. incognita* and *F. oxysporum* on cowpea by the application of neem cake and carbofuran in various combinations. They reported that the treatment with the reduced doses of both neem cake @ 0.5 w/w and carbofuran @ 0.75 kg a.i./ha were the best in checking the severity of disease and improving plant vigour followed by neem cake alone or at it full dose. The carbofuran alone however, did not show any remarkable effect on reducing the disease complex.

Haseeb and Shukla (2002) evaluated the effect of some bio-pesticides, bio-agents and chemicals for the management of wilt complex of chickpea cv. Desi T3 caused by *F. oxysporum* f. sp. *ciceri* and *M. incognita* under field conditions. They reported that the seed treatment with dimethoate 30 EC (0.8%

v/w), chlorpyriphos (1% v/w), triazophos (3% v/w), neemark (5% v/w), neem seed powder (5% w/w), spore suspension (10^8 spore/ml) of *P. lilacinus* and *A. niger* (2% v/w), latex of *Calotropis procera* (1% v/w) and soil application of neem seed powder (50 kg/ha) and carbofuran (2 kg a.i./ha) resulted in the reduction of wilt incidence. However, the observations recorded at harvest on the effect of seed treatments on nematode population in roots and soil, root-knot index, percent galled area in roots, root infection, was found to be non-significant. Whereas, the treatment of seeds with neem seed powder had a little effect on the above parameters till the end of experiment. On the basis of grain yield, carbofuran was the most effective in increasing the yield, followed by neem seed powder as soil treatment and seed treatment, neemark, *P. lilacinus, A. niger,* dimethoate, chlorpyriphos, latex and triazophos, respectively.

More recently, Haseeb (2003) evaluated the efficacy of various organic amendments and bio-control agents for the management of wilt complex of brinjal, tomato and chilli caused by *F. oxysporum* and *M. incognita*. He reported that application of carbofuran @ 1.0 kg a.i./ha + topsin-M @ 2.0 kg/ha, or carbofuran (0.5 kg a.i./ha) + neem seed powder @ 1.0 q/ha or carbofuran/neem seed powder + spore suspension of (10^8 spores/ml) of *T. harzianum, T. viride/P. lilacinus* or *A. niger* @ 50 l/ha 7-21day-before sowing/transplanting, resulted significant decrease in the incidence of wilt and increase in yield. In field also the pre sowing application of carbofuran @ 33.0 kg/ha + topsin-M @ 1.0 kg/ha, or carbofuran + neem seed powder @ 1.0 q/ha or carbofuran/neem seed powder + *T. harzianum* @ 50.0 kg/ha (10^8 spores/g) provided the satisfactory management of this disease.

Haseeb and Shukla (2004) determined the effect of pigeonpea seed treatment with *P. lilacinus, A. niger* and *T. harzianum* @ 0.1 and 0.2% v/w (spore suspension with 10^8 spores/ml) and neem seed powder @ 5% w/w under field conditions. They have significant suppression of wilting of plants and plant-parasitic nematodes.

To investigate the efficacy of pesticides, neem seed powder and bio-control agents on *M. incognita* and *S. sclerotiorum* disease complex, experiments were conducted in pots (10a) and in an experimental field (10b).

10a. Comparative efficacy of pesticides, neem seed powder and bio-control agents on disease development, nematode multiplication, growth and oil yield in *M. incognita* and *S. sclerotiorum* inoculated plants of *M. arvensis* cv. Gomti

To determine the effect of various treatments and treatment combinations on the disease complex of *M. incognita* and *S. sclerotiorum* on *M. arvensis* cv. Gomti in pots following procedures were applied.

MATERIALS AND METHODS

10a.1 Application of treatments

The preparation of pots and procedure for the application of treatments was done in the same manner as described in 8.2.

10a.2 Transplanting and inoculation

A single sucker was planted into each treated pot and 5,000 J2 of *M. incognita* and 3 g mycelium of *S. sclerotiorum*/5 kg soil were inoculated simultaneously into each pot. The procedure for transplanting and inoculation was same as described earlier in 2.1 and 4.1.

For each treatment there were four replicates. Four inoculated pots were left untreated and four pots were left uninoculated. The experiment was laid out as a completely randomized block design.

10a.3 Recording of data

Recording of data regarding plant growth, oil yield, nematode population in soil and in roots/suckers, root-knot index and percent root infection by fungus was done in the same manner as mentioned earlier in 2.4.1, 2.4.6, 1.5.1, 1.5.2, 1.3 and 1.4, respectively.

RESULTS

In general, application of all the treatments and treatment combinations had appreciable increase in plant growth i.e. height, fresh and dry weights, and oil yield of the plants as compared to untreated inoculated plants (Table 21; Fig. 19). All the treatments were also able to reduce the root-knot development, *M. incognita* reproduction rate and roots/suckers infection by *S. sclerotiorum* (Fig. 20).

The reduction in shoot height, shoot fresh weight, roots/suckers fresh weight, shoot dry weight, roots/suckers dry weight and oil yield was observed least in plants treated with neem seed powder + carbofuran and carbofuran + carbendazim as compared to untreated uninoculated plants (Plate 18). The maximum reduction in nematode reproduction rate (1.39) and root-knot index (0.15) was achieved in neem seed powder + carbofuran treated plants. The maximum reduction in roots/suckers infection due to fungus was achieved in neem seed powder + carbendazim and carbendazim alone treated plants. The highest reproduction rate, root-knot index and percent roots/suckers infection by fungus (13.95, 1.62 and 60.0%, respectively) was observed in untreated inoculated plants.

Plant dry weight data indicate that the best treatment for improving plant weight was ($P \leq 0.01$) found in plants treated with neem seed powder + carbofuran/carbendazim + carbofuran followed by neem seed powder + carbendazim, carbofuran alone/neem seed powder alone/neem seed powder + *P. lilacinus*/neem seed powder + *T. harzianum*/neem seed powder + *P. fluorescens, P. lilacinus* + *T. harzianum, P. fluorescens* + *P. lilacinus, P. fluorescens* + *T. harzianum, P. lilacinus* alone/*T. harzianum* alone, *P. fluorescens* alone and carbendazim alone, respectively. Similarly, oil yields indicated that neem seed powder + carbofuran and carbendazim + carbofuran were the best treatements ($P \leq 0.01$) in improving oil yield followed by neem seed powder + carbendazim/carbofuran alone/neem seed powder alone/neem seed powder + *P. lilacinus*/neem seed powder + *T. harzianum,* neem seed powder + *P. fluorescens*/neem seed powder + *T. harzianum/P. fluorescens* + *T. harzianum, P.*

Table 21: Comparative efficacy of pesticides, neem seed powder and bio-control agents on disease development, nematode multiplication, growth and oil yield in *Meloidogyne incognita* (5000 J2/5 kg soil) and *Sclerotinia sclerotiorum* (3 g mycelium/5 kg soil) inoculated plants of *Mentha arvensis* cv. Gomti.[a]

Treatments	Shoot height (cm)	Plant fresh weight (g)			Plant dry weight (g)			Final nematode population			Reproduction factor	[b]Root-knot index	[c]Roots & suckers infection	Oil yield (ml/100 g fresh herb)
		Shoot	Roots & suckers	Total	Shoot	Roots & suckers	Total	Roots & suckers	Soil (5 kg)	Total				
Untreated uninoculated control	75.0	148.0	135.0	283.0	35.4	26.0	61.4	-	-	-	-	-	-	0.76
Untreated inoculated	53.2 (29.1)	80.0 (46.6)	66.2 (51.0)	146.2	19.0 (46.3)	12.5 (51.9)	31.5	31776	38000	69776	13.95	1.62	60.00	0.50 (34.2)
Carbofuran	71.3 (4.9)[d]	138.5 (6.4)	119.0 (11.8)	257.5	33.0 (6.8)	23.0 (11.5)	56.0	3570	4000	7570	1.51	0.44	20.00	0.70 (7.9)
Carbendazim	64.5 (14.0)	106.4 (28.1)	91.7 (32.1)	198.1	25.3 (28.5)	17.5 (32.7)	42.8	29344	30000	59344	11.87	1.15	10.00	0.58 (23.7)
Neem seed powder	71.2 (5.1)	138.0 (6.7)	118.7 (12.1)	256.7	33.0 (6.8)	22.9 (11.9)	55.9	4748	6000	9748	2.15	0.37	18.00	0.69 (9.3)
P. fluorescens	62.0 (17.3)	108.1 (27.0)	95.7 (29.1)	203.8	25.5 (28.0)	18.2 (30.0)	43.7	19140	20000	39140	7.83	0.87	35.00	0.60 (21.0)
P. lilacinus	63.8 (14.9)	113.7 (23.2)	97.0 (28.1)	210.7	27.0 (23.7)	18.5 (28.8)	45.5	17460	18000	35460	7.09	0.70	38.00	0.60 (21.0)
T. harzianum	64.5 (14.0)	112.0 (24.3)	96.2 (28.7)	208.2	26.7 (24.6)	18.5 (28.8)	45.2	18278	20000	38278	7.65	0.75	32.00	0.60 (21.0)
Neem seed powder + Carbofuran	74.8 (0.3)	145.0 (2.0)	131.0 (3.0)	275.8	34.5 (2.5)	25.2 (3.1)	59.7	3930	3000	6930	1.39	0.15	15.00	0.76 (0.0)
Neem seed powder + Carbendazim	73.2 (2.4)	140.7 (4.9)	125.5 (7.0)	266.2	33.5 (5.4)	24.0 (7.7)	57.5	5020	6000	11020	2.20	0.40	8.0	0.70 (7.9)
Neem seed powder + *P. fluorescens*	69.5 (7.3)	134.5 (9.1)	116.0 (14.1)	250.5	32.0 (9.6)	22.2 (14.6)	54.2	12760	12000	24760	4.95	0.55	25.00	0.65 (14.5)
Neem seed powder + *P. lilacinus*	71.0 (5.3)	137.6 (7.0)	118.5 (12.2)	256.1	32.8 (7.3)	22.6 (13.1)	55.4	10665	10000	20665	4.13	0.45	24.00	0.68 (10.5)
Neem seed powder + *T. harzianum*	72.0 (4.0)	136.0 (8.1)	117.6 (13.1)	253.6	32.3 (8.7)	22.4 (13.8)	54.7	11760	10000	20760	4.35	0.50	25.00	0.68 (10.5)
P. fluorescens + *P. lilacinus*	65.0 (13.3)	124.3 (16.0)	108.0 (20.0)	232.3	29.5 (16.7)	20.5 (21.1)	50.0	162.00	17000	33200	6.64	0.60	32.00	0.63 (17.1)
P. fluorescens + *T. harzianum*	64.6 (13.9)	120.0 (18.9)	102.5 (24.1)	222.5	28.5 (19.5)	19.7 (24.2)	48.2	15400	18000	34400	6.88	0.62	28.00	0.63 (17.1)
P. lilacinus + *T. harzianum*	65.2 (13.2)	124.6 (15.8)	114.0 (15.0)	232.6	29.5 (16.5)	21.8 (16.1)	51.3	13680	16000	29680	5.94	0.55	26.0	0.65 (14.5)
Carbofuran + Carbendazim	73.8 (1.6)	145.0 (2.0)	130.2 (3.5)	275.2	34.3 (3.8)	25.0 (3.8)	59.3	5208	5000	10208	2.04	0.25	8.00	0.75 (1.3)
L.S.D. $_{0.05}$	3.4	4.5	3.3	4.5	1.9	0.5	0.9	213.0	2085.0	2098.0	0.12	0.07	2.9	0.03
L.S.D. $_{0.01}$	4.6	6.2	4.5	6.1	2.6	0.6	1.2	288.0	2814.0	2832.0	0.16	0.09	3.9	0.04

[a]Each value is an average of four replicates.
[b]Root-knot index: 0 = 0%, 1 = 1-25%, 2 = 26-50%, 3 = 51-75%, 4 = 76-100%.
[c]Percent roots and suckers infection by *S. sclerotiorum*.
[d]Figures in parentheses are percent reduction over untreated uninoculated control.

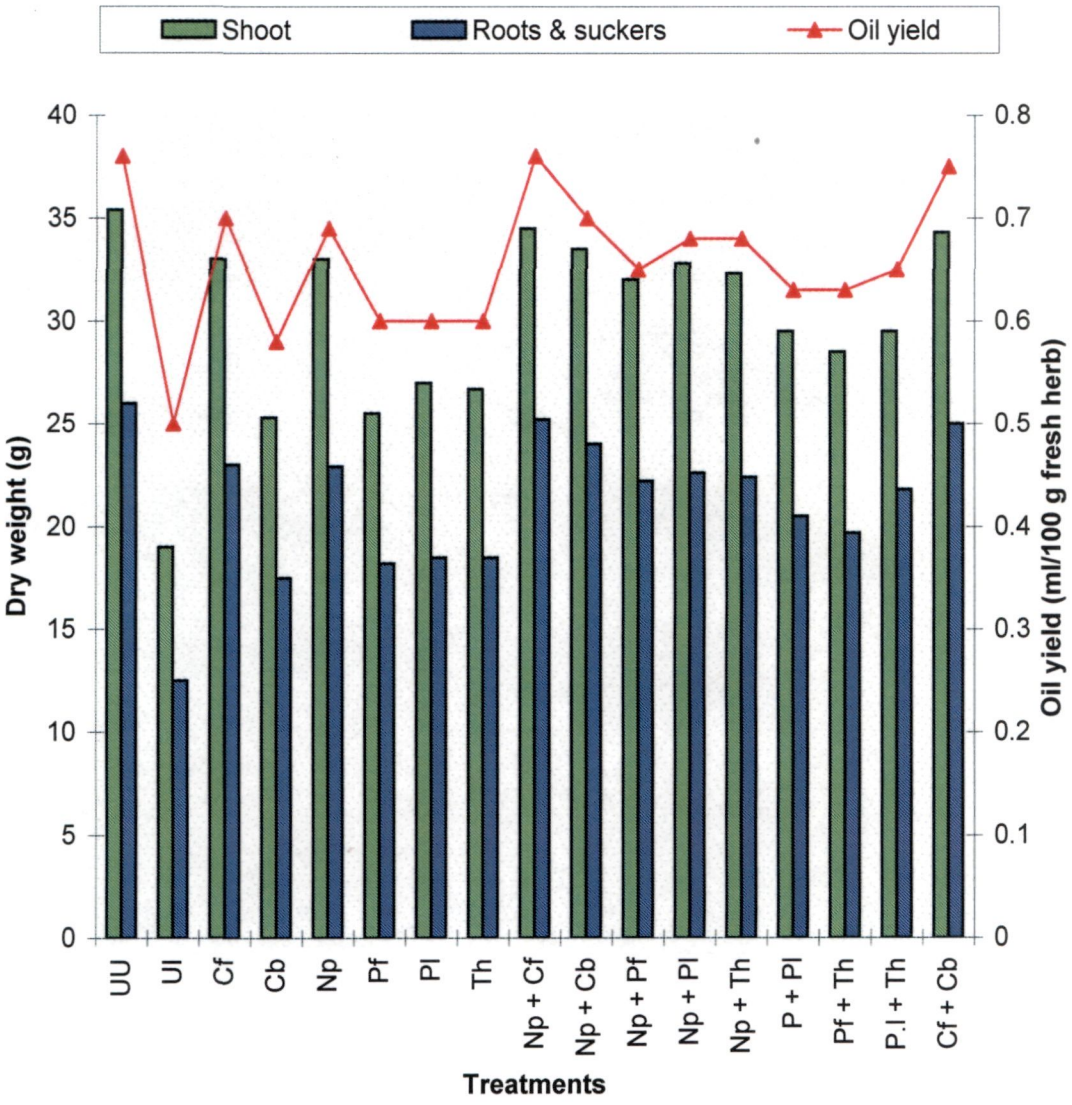


Fig. 19: Comparative efficacy of various treatments on the growth and oil yield of *M. incognita* (5000 J2/5 kg soil) and *S. sclerotiorum* (3 g mycelium/5 kg soil) inoculated plants of *M. arvensis* cv. Gomti.

UU = Uninoculated untreated
Cf = Carbofuran
Cb = Carbendazim
Np = Neem seed powder
Pl = *P. lilacinus*
Pf = *P. fluoroscens*
Th = *T. harzianum*

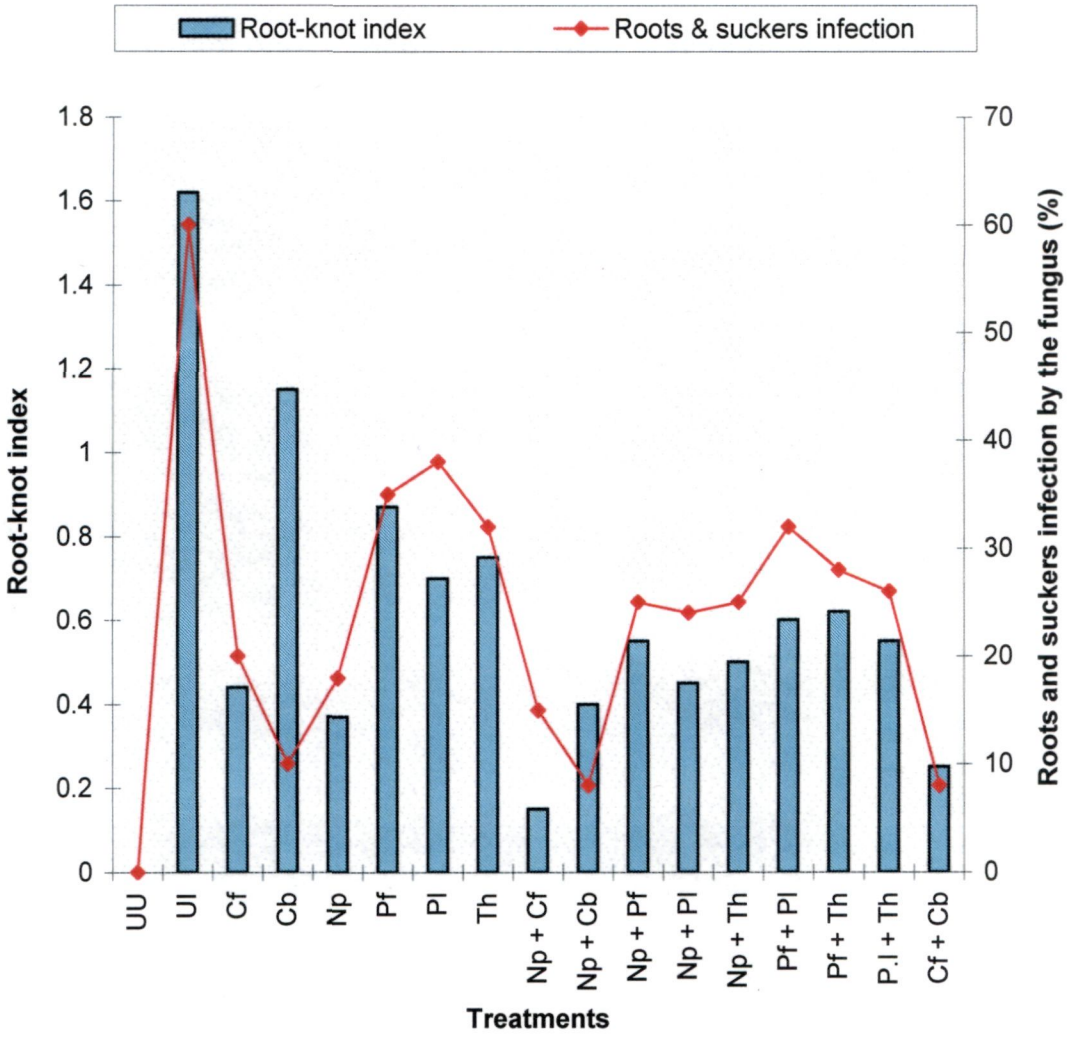


Fig. 20: Comparative efficacy of various treatments on disease development in *M. incognita* (5000 J2/5 kg soil) and *S. sclerotiorum* (3 g mycelium/ 5 kg soil) inoculated plants of *M. arvensis* cv. Gomti.

Root-knot index:- 0 = 0%, 1 = 1-25%, 2 = 26-50%, 3 = 51-75%, 4 = 76-100%

UU = Uninoculated untreated Cf = Carbofuran Cb = Carbendazim
Np = Neem seed powder PI = *P. lilacinus* Pf = *P. fluoroscens*
Th = *T. harzianum*

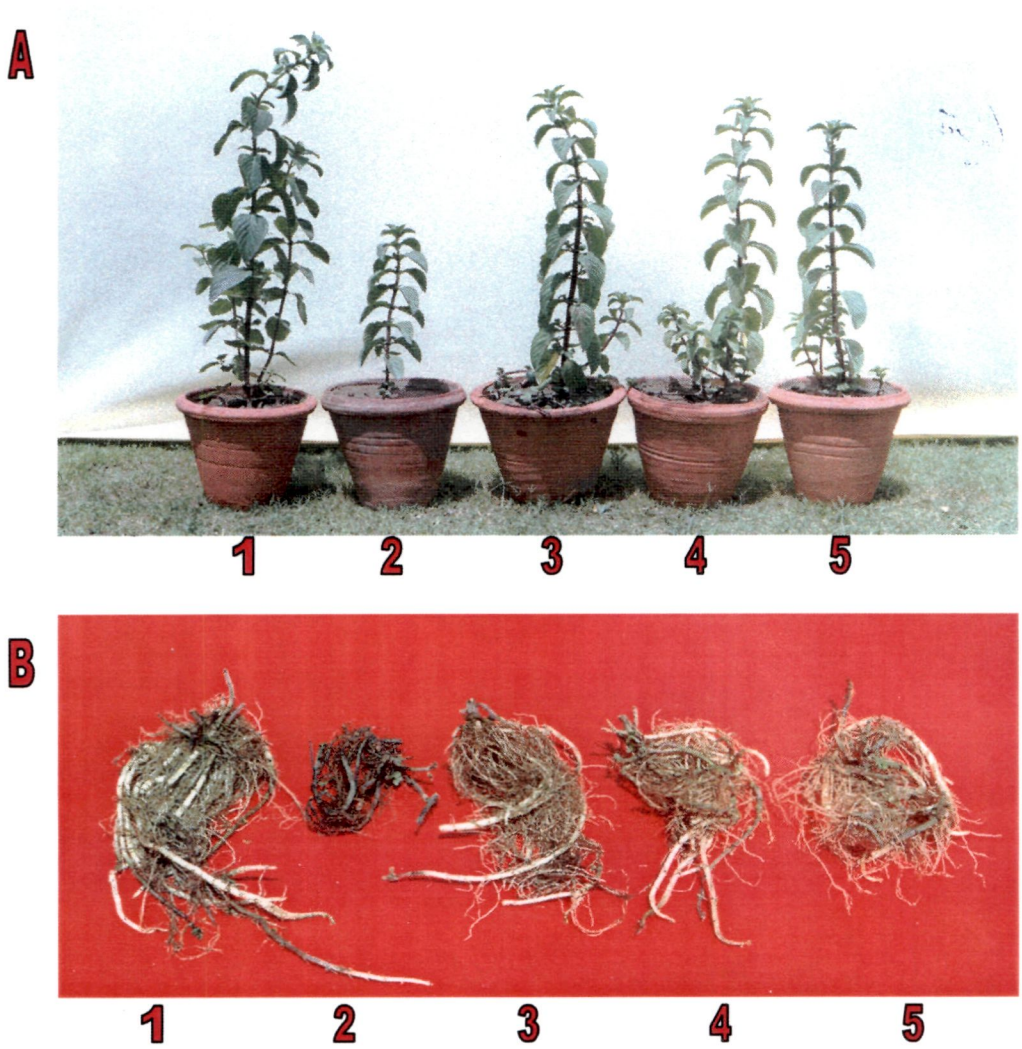


Plate 18: Effect of various treatments on aerial growth (A) and roots and suckers development (B) of *M. arvensis* cv. Gomti inoculated with *M. incognita* (5000 J2/pot) and *S. sclerotiorum* (3 g mycelium/pot). Showing the best three treatments.

1 = Uninoculated untreated
2 = Inoculated untreated
3 = Neem seed powder @ 250 mg/kg soil + carbofuran @ 0.75 mg a.i./kg soil
4 = Carbofuran @ 0.75 mg a.i./kg soil + carbendazim @ 0.5 mg a.i./kg soil
5 = Neem seed powder @ 250 mg/kg soil + carbendazim @ 0.5 mg a.i./kg soil

lilacinus alone/*T. harzianum* alone/*P. fluorescens* alone/carbendazim alone, respectively. Analyses for reproduction rate of nematode shows that neem seed powder + carbofuran was the best treatment for reducing nematode population ($P \leq 0.01$) followed by carbofuran alone, carbendazim + carbofuran/neem seed powder alone/neem seed powder + carbendazim, neem seed powder + *P. lilacinus,* neem seed powder + *T. harzianum;* neem seed powder + *P. fluorescens, P. lilacinus* + *T. harzianum, P. fluorescens* + *P. lilacinus* alone, *P. fluorescens* + *T. harzianum* alone, *P. lilacinus, T. harzianum, P. fluorescens* alone and carbendazim alone, respectively. Analyses for percent roots/suckers infection due to fungus indicates that neem seed powder + carbendazim/carbendazim alone/carbendazim + carbofuran were the best treatments ($P \leq 0.01$) for decreasing roots/suckers infection by fungus, followed by neem seed powder + carbofuran, neem seed powder alone/carbofuran alone, neem seed powder + *P. lilacinus*/neem seed powder + *T. harzianum*/neem seed powder + *P. fluorescens, P. lilacinus* + *T. harzianum/P. fluorescens* + *T. harzianum/P. fluorescens* + *P. lilacinus/T. harzianum* alone, *P. fluorescens* alone/*P. lilacinus* alone, respectively.

DISCUSSION

The results regarding the management of *M. arvensis* cv. Gomti inoculated with *M. incognita* and *S. sclerotiorum* showed that in general all the treatments were able to improve the plant growth and oil yield as compared to untreated inoculated plants. All treatments were also able to reduce the root-knot disease development, nematode reproduction rate and roots and suckers infection due to fungus. The most satisfactory result was provided by the neem seed powder + carbofuran treatment followed by carbofuran + carbendazim, neem seed powder + carbendazim, carbofuran alone, neem seed powder alone, neem seed powder + *P. lilacinus*, neem seed powder + *T. harzianum*, neem seed powder + *P. fluorescens*, *P. lilacinus* + *T. harzianum, P. lilacinus* alone, *T. harzianum* alone, *P. fluorescens* alone and carbendazim alone respectively.

The present study clearly revealed that all treatments applied alone or in combinations were able to improve the plant growth and oil yield, and reduced

the root-knot development and roots/suckers infection due to fungus. However, the treatments involving two managing components were observed to be more effective than the corresponding alone treatments. It appeared that individual components of the combinations treatment made a cumulative multiple and differential effect on the pathogens which they failed to sustain and were never allowed to develop at such a level that could cause serious damage to plants.

10b. The comparative efficacy of pesticides, neem seed powder and bio-control agents on disease development, nematode multiplication, growth and oil yield in plants of *M. arvensis* cv. Gomti grown in a field naturally infested with *M. incognita* and *S. sclerotiorum*.

To investigate the efficacy of various treatments under field conditions the experiment was conducted at experimental field of Faculty of Agricultural sciences, A.M.U., Aligarh.

MATERIALS AND METHODS

10b.1 Preparation and maintenance of sick field

In order to prepare the sick field, 100 J2 of *M. incognita* and 1.5 g mycelium of *S. sclerotiorum* were inoculated/m of furrow made for transplanting of *M. arvensis*. In order to maintain infestation levels the field was continuously cropped with *M. arvensis* for 24 months.

10b.2 Preparation of plots in an infested field and application of various treatments

The experimental field was ploughed and after levelling, plots of 3 x 4 m^2 were prepared. In well-prepared plots, furrows were made 30 cm apart and various treatment materials were manually applied in furrow before transplanting. There were three replicates for each treatment. The experiment was laid out as a completely randomized block design.

The application of various treatments was done according to the scheme mentioned below:

S. No.	Treatments	Rate of application/ha	Rate of application/plot
1	Untreated control	–	–
2	Carbofuran (Furadan 3G)	3.0 kg a.i.	3.6 g a.i.
3	Carbendazim (Bavistin 50%)	2.0 kg a.i.	2.4 g a.i.
4	Neem seed powder	100 kg	120 g
5	*Pseudomonas fluorescens* (10^8cfu/g)	100 kg	120 g
6	*Paecilomyces lilacinus* (10^8cfu/g culture)	100 kg	120 g
7	*Trichoderma harzianum* (10^8cfu/g culture)	100 kg	120 g
8	Neem seed powder + Carbofuran	*	*
9	Neem seed powder + Carbendazim	*	*
10	Neem seed powder + *P. fluorescens*	*	*
11	Neem seed powder + *P.lilacinus*	*	*
12	Neem seed powder + *T. harzianum*	*	*
13	*P. lilacinus* + *P. fluorescens*	*	*
14	*P. fluorescens* + *T. harzianum*	*	*
15	*P. lilacinus* + *T. harzianum*	*	*
16	Carbofuran + Carbendazim	*	*
17	Fallow	-	-

* In all combined treatments, rate of application was reduced to half of standard rate.

10b.3 Transplanting of suckers

After application of various treatments, 300 g suckers of *M. arvensis* cv. Gomti were planted head to head into each plot at row spacing of 30 cm. The plots were irrigated after planting of suckers, and as needed during the season.

10b.4 Recording of data

After 110 days the fresh weight of herb in each plot was determined. Estimation of oil yield, nematode population in soil and roots/suckers, root-knot index and percent roots/suckers infection by fungus was done in the same manner as described in 2.4.6, 1.5.1, 1.5.2, 1.3 and 1.4, respectively.

RESULTS

In general all treatments were able to improve the plant growth and oil yield as compared to untreated control (Table 22; Fig.21). The maximum percent increase in shoot fresh weight, shoot dry weight and oil yield was observed in plots treated with neem seed powder + carbofuran, carbendazim + carbofuran, neem seed powder + carbendazim, carbofuran alone, neem seed powder alone (Plate 19). All treatments were also observed to decrease the nematode reproduction, root-knot index and percent roots/suckers infection by *S. sclerotiorum* (Fig. 22). The lowest *M. incognita* reproduction rate (1.18) and root-knot index (0.50) was achieved by neem seed powder + carbofuran treatment, whereas, roots/suckers infection by fungus decreased maximum (10.0%) by carbendazim alone treatment.

Analyses of data for shoot dry weight indicated that neem seed powder + carbofuran, carbofuran + carbendazim, neem seed powder + carbendazim, carbofuran alone and neem seed powder alone were superior ($P \leq 0.01$) than the neem seed powder + *P. lilacinus,* neem seed powder + *T. harzianum*, neem seed powder + *P. fluorescens, P. lilacinus* + *T. harzianum* followed by *P. fluorescens* + *P. lilacinus, P. fluorescens* + *T. harzianum/T. harzianum* alone, *P. lilacinus* alone, *P. fluorescens* alone. Carbendazim alone was found inferior to all the treatments.

Analyses of data for oil yield indicated that neem seed powder + carbofuran, carbofuran + carbendazim, neem seed powder + carbendazim, carbofuran alone and neem seed powder alone were equally ($P \leq 0.01$) most effective and superior to neem seed powder + *P. lilacinus*, neem seed powder + *T. harzianum*, neem seed powder + *P. fluorescens, P. lilacinus* + *T. harzianum* followed by *P. fluorescens* + *P. lilacinus, P. fluorescens* + *T. harzianum, T. harzianum* alone, *P. lilacinus* alone, *P. fluorescens* alone and carbendazim alone.

Neem seed powder + carbofuran and carbendazim + carbofuran were equally ($P \leq 0.01$) most effective in suppressing the nematode reproduction followed by neem seed powder alone, carbofuran alone, neem seed powder +

Table 22: Comparative efficacy of pesticides, neem seed powder and bio-control agents on disease development, nematode multiplication, growth and oil yield in plants of *M. arvensis* cv. Gomti grown in a field naturally infested with *M. incognita* and *S. sclerotiorum* (4 x 3m^2). [a]

Treatments	Shoot weight (kg/plot)		Oil yield (ml/100 g fresh herb)	Final nematode population			Reproduction factor	[b]Root-knot index	[c]Roots & suckers infection
	Fresh	Dry		Roots&suckers (per g)	Soil (250 g)	Total			
Untreated control	21.3	5.3	0.60	500	2800	3300	8.25	3.00	55.0
Carbofuran	26.5 (24.4)[d]	6.6 (24.5)	0.72 (20.0)	120	700	820	2.05	0.75	35.0
Carbendazim	22.4 (5.2)	5.6 (5.7)	0.63 (5.0)	480	2600	3080	7.7	2.75	10.0
Neem seed powder	26.2 (23.0)	6.5 (22.6)	0.70 (16.7)	120	700	820	2.05	0.75	15.0
P. fluorescens	23.6 (10.8)	5.9 (11.3)	0.64 (6.7)	340	2000	2340	5.85	2.00	45.0
P. lilacinus	24.5 (15.0)	6.1 (15.1)	0.65 (8.3)	300	1500	800	4.50	1.22	40.0
T. harzipanum	24.0 (12.7)	6.0 (13.2)	0.65 (8.3)	313	1600	913	4.70	1.26	34.0
Neem seed powder + Carbofuran	27.2 (27.7)	6.8 (28.3)	0.74 (23.3)	73	400	473	1.18	0.50	20.0
Neem seed powder + Carbendazim	26.8 (25.8)	6.7 (26.4)	0.73 (21.7)	113	800	913	2.28	0.80	12.0
Neem seed powder + *P. fluorescens*	25.7 (20.6)	6.4 (20.7)	0.66 (10.0)	240	1200	1440	3.60	1.10	25.0
Neem seed powder + *P. lilacinus*	26.0 (22.1)	6.5 (22.6)	0.68 (13.3)	187	1000	1187	2.97	1.00	20.0
Neem seed powder + *T. harzianum*	25.9 (21.6)	6.4 (20.7)	0.68 (13.3)	200	1000	1200	3.00	1.00	23.0
P. fluorescens + *P. lilacinus*	24.8 (16.4)	6.1 (15.1)	0.65 (8.3)	260	1360	1560	3.90	1.15	34.0
P. lilacinus+*T. harzianum*	25.2 (18.3)	6.8 (18.9)	0.60 (10.0)	260	1300	1560	3.90	1.15	28.0
P. fluorescens+ *T. harzianum*	24.8 (16.4)	6.2 (17.0)	0.65 (8.3)	287	1400	1687	4.22	1.22	30.0
Carbofuran + Carbendazim	27.0 (26.8)	6.7 (26.4)	0.74 (23.3)	60	500	560	1.40	0.65	15.0
L.S.D. $_{0.05}$	2.3	0.16	0.04	35.5	213.7	1035.6	0.75	0.09	6.83
L.S.D. $_{0.01}$	3.2	0.21	0.05	47.9	288.4	1397.6	1.01	0.13	9.22

[a]Each value is an average of three replicates.
[b]Root-knot index : 0 = 0%, 1 = 1-25%, 2 = 26-50%, 3 = 51-75%, 4 = 76-100%.
[c]Percent roots and suckers infection by *S. sclerotiorum*.
[d]Figures in parentheses are percent increase over untreated control.
Pi of *Meloidogyne* spp. = 200 (± 25) J2/250 g soil).

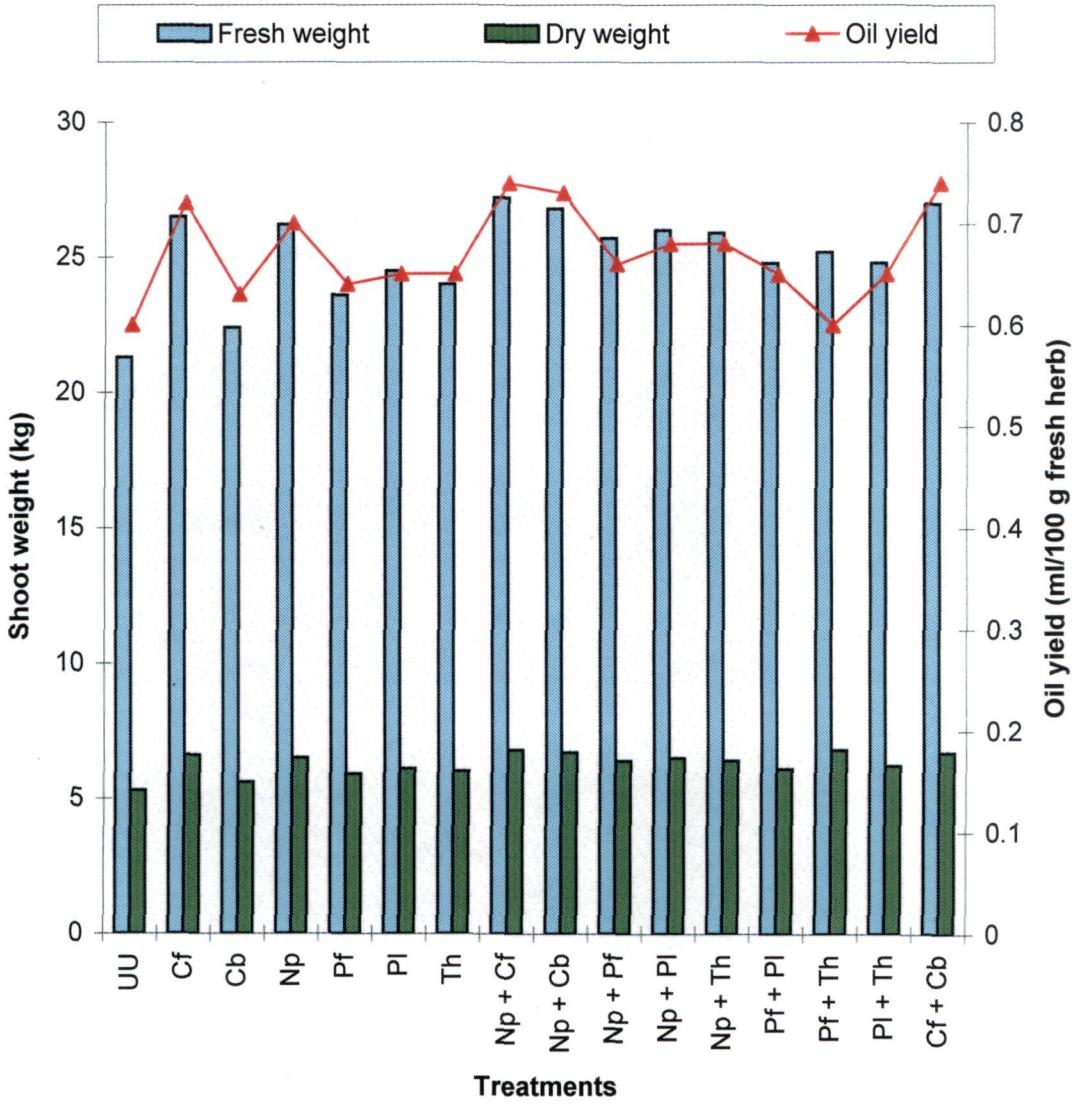


Fig. 21 : Comparative efficacy of various treatments on growth and oil yield of *M. arvensis* cv. Gomti grown in sick field.

UU = Uninoculated untreated
Cf = Carbofuran
Cb = Carbendazim
Np = Neem seed powder
Pl = *P. lilacinus*
Pf = *P. fluoroscens*
Th = *T. harzianum*

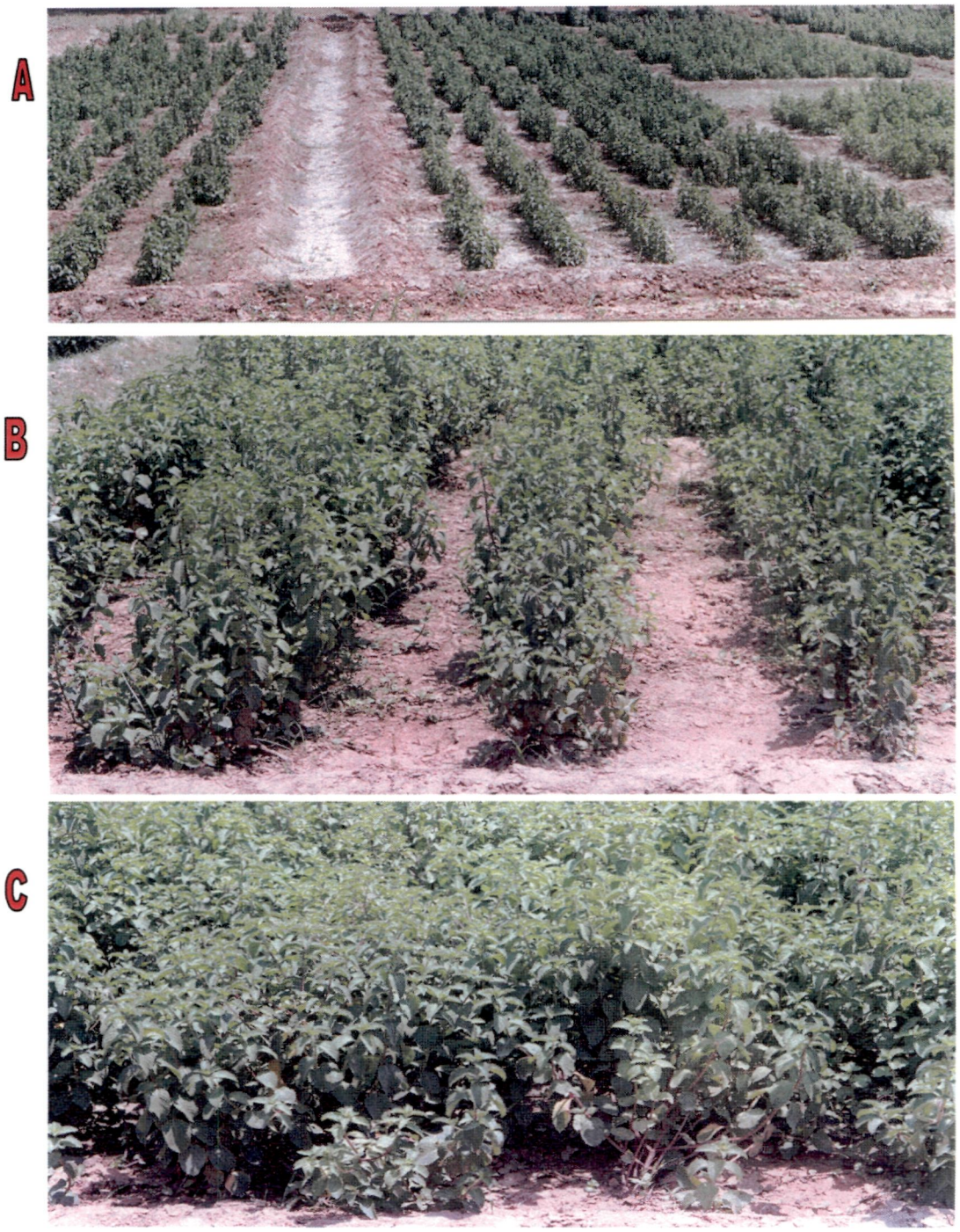


Plate 19: Effect of various treatments on the growth of *M. arvensis* cv. Gomti grown in a field infested with *M. incognita* and *S. sclerotiorum*.

A: A view of the experimental field.
B: Untreated plot.
C: Plot treated with neem seed powder @ 50 kg/ha + carbofuran @1.5 kg a.i./ha (most effective treatment).

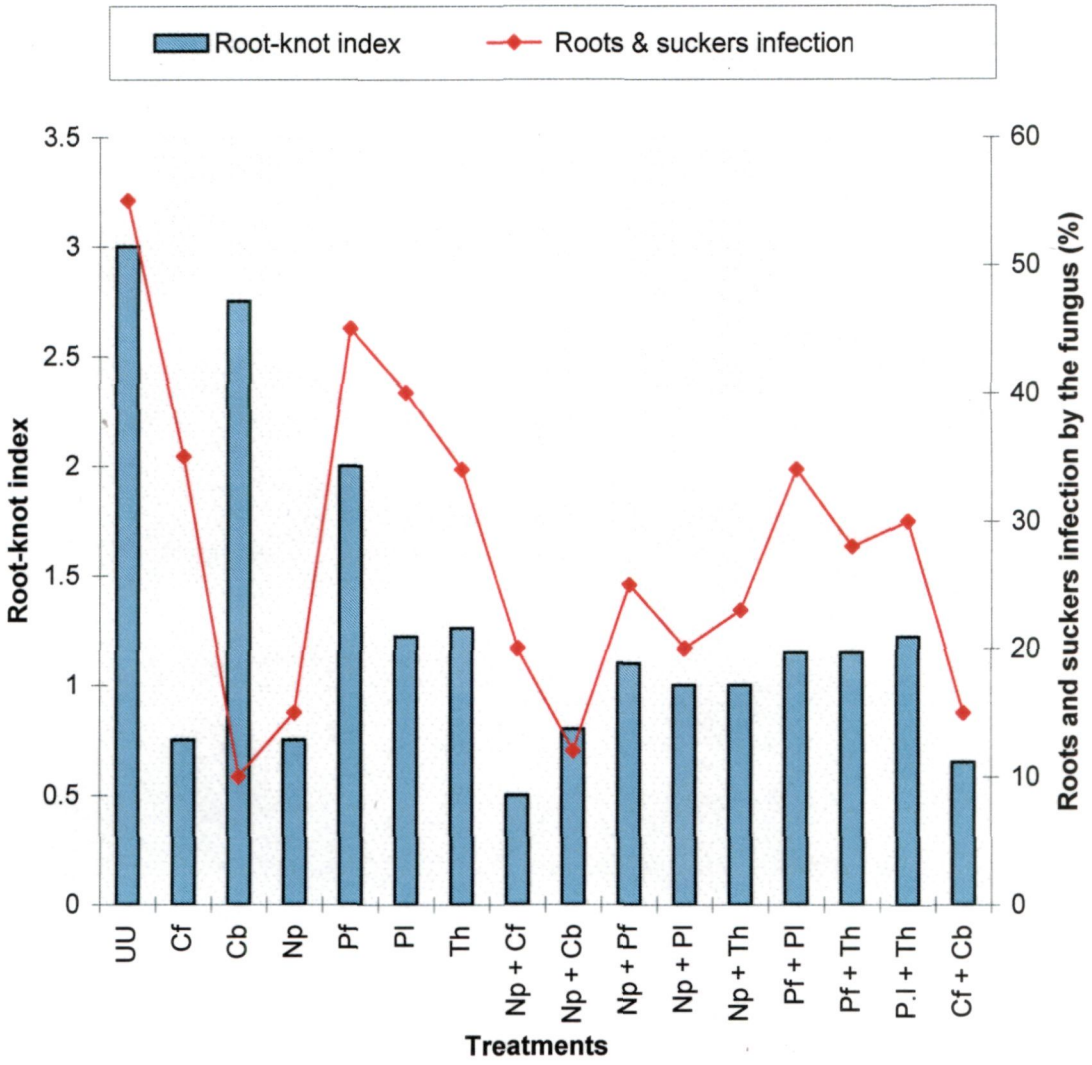


Fig. 22: Comparative efficacy of various treatments on disease development in plants of *M. arvensis* cv. Gomti grown in sick field.

Root-knot index: 0 = 0%, 1 = 1-25%, 2 = 26-50%, 3 = 51-75%, 4 = 76-100%.

UU = Uninoculated untreated Cf = Carbofuran Cb = Carbendazim
Np = Neem seed powder Pf = *P. fluoroscens* Pl = *P. lilacinus*
Th = *T. harzianum*

carbendazim, neem seed powder + *P. lilacinus,* neem seed powder + *T. harzianum,* neem seed powder + *P. fluorescens, P. fluorescens* + *P. lilacinus, P. lilacinus* + *T. harzianum, P. fluorescens* + *T. harzianum, P. lilacinus* alone, *T. harzianum* alone, *P. fluorescens* alone and carbendazim alone, respectively.

Analyses of data ($P \leq 0.01$) for percent roots/suckers infection by fungus indicates that, all the treatments were able to give satisfactory reduction in roots/suckers infection due to fungus (Table 22). Carbendazim alone and neem seed powder + carbendazim were best followed by neem seed powder alone/carbofuran + carbendazim, neem seed powder + *P. lilacinus*/neem seed powder + carbofuran/neem seed powder + *T. harzianum,* neem seed powder + *P. fluorescens/P. lilacinus* + *T. harzianum*/carbofuran alone, *P. fluorescens* + *T. harzianum, P. fluorescens* + *P. lilacinus, T. harzianum, P. lilacinus* alone and *P. fluorescens* alone respectively.

DISCUSSION

The results of comparative efficacy of pesticides, neem seed powder and bio-control agent against *M. incognita* and *S. sclerotiorum* carried out in experimental field showed that the results were similar to the findings of previous experiment conducted in pots. Therefore, it further confirms the positive efficacy of all treatments against this disease complex. Several workers have also achieved the success in managing the disease complex, involving nematode and fungus by utilizing various treatments (Parveen *et al.*, 1993; Latha *et al.,* 2000; Haseeb and Shukla, 2002, 2004; Haseeb, 2003).

Four experiments carriedout to determine the comparative efficacy of different treatments against *M. incognita-S. sclerotiorum* infecting *M. arvensis* cv. Gomti under pot and field conditions indicated that few of them were highly effective. Among various treatments, application of neem seed powder @ 100 kg/ha was best in managing the pathogens and increasing the yield. Carbofuran (3 kg a.i./ha) increased herb and oil yield and suppressed *M. incognita* upto considerably high extent but could not prevent roots and suckers from *S. sclerotiorum* infection. Carbendazim (2 kg a.i./ha) could only prevent *S.*

sclerotiorum infection in roots without considerable increase in yield and suppression of the nematode. Bio-control agents could not prove their potential. However, when two treatment materials at half doses were combined better results were achieved. Combination of carbofuran (1.5 kg a.i./ha) or carbendazim (1.0 kg a.i./ha) with neem seed powder (50 kg/ha) provided highly encouraging results. Biocontrol agents also performed better with neem seed powder.

On the basis of results the application of neem seed powder (50 kg/ha) + carbofuran (1.5 kg a.i./ha) or carbendazim (1.0 kg a.i./ha) is recommended for getting best results. However, to avoid the use of pesticides, application of neem seed powder (100 kg/ha) or neem seed powder (50 kg/ha) + *T. harzianum/P. lilacinus/P. fluorescens* (50 kg culture/ha) may be recommended.

Summary

SUMMARY

Japanese mint, *Mentha arvensis* L. is a rich source of menthol, a chemical, which is widely used in pharmaceutical, flavouring and cosmetic industries. It is cultivated on a large scale in tropical and sub-tropical countries of the world. Besides other pathogens, fungi and plant-parasitic nematodes causes considerable damage to it. Keeping in view the importance of *M. arvensis*, an ever-increasing demand for menthol, and the damaging potential of root-knot nematodes alone and in combination with several soil borne fungi, attempts were made to generate information pertaining to survey of mint growing areas in Uttar Pradesh for the association of plant-parasitic nematodes and soil borne fungi infecting Japanese mint, singular and combined effect of *Meloidogyne incognita* and *Sclerotinia sclerotiorum* on growth and oil yield of *M. arvensis* cv. Gomti, factors effecting (soil type and pH) the severity of disease and measures to manage the losses due to these pathogens.

Survey carried out in districts of Aligarh, Badaun, Bareilly, Bulandshahar, Etah, Moradabad and Rampur indicated that during initial stages of plant growth in March, visually there were no specific symptoms of nematode attack. The water soaked areas on suckers due to *S. sclerotiorum* were frequently noticed. At crop maturity in May, patches of diseased plants showed symptoms of stunting with chlorotic and smaller leaves. Roots/suckers of such plants had severe galling with shiny egg masses. At several places, the roots/suckers were dark brown to black in colour and many were rotting. At various locations black coloured sclerotia of *S. sclerotiorum* were also found attached to the infected suckers.

In general, *Meloidogyne* spp. J2 was the dominant population in the rhizosphere of *M. arvensis*. However, in many samples either *Tylenchorhynchus* spp., *R. reniformis* or *P. thornei* dominated the population. Among other plant-parasitic nematodes, *Hel. indicus* and *Hop. indicus* were found consistently in higher numbers, while *Tylenchus* sp., *Xiphinema* sp., *Longidorous* sp. and *Criconimoides* sp. were found occasionally. On the examination of perineal

patterns of mature females excised from the roots/suckers of *M. arvensis,* collected from each and every locality during survey revealed that *M. incognita* (70%) was more prevalent than *M. javanica* (30%).

At all localities roots/suckers of *M. arvensis* were mainly infected with *S. sclerotiorum*, though in some cases other soil borne fungi were also found infecting the roots/suckers of *M. arvensis.* Fungi isolated from infected roots and suckers were *Fusarium pallidoroseum, F. solani* and *Rhizoctonia solani, S. sclerotiorum.* The highest population of *Meloidogyne* spp., root-knot index and percent root/suckers infection by fungi was found in Moradabad followed by Bulanshahar, Aligarh, Badaun, Bareilly, Rampur and Etah districts, respectively.

The pathogenicity tests of *M. incognita* on *M. arvensis* cv. Gomti evidenced its potentiality in reducing the shoot height, shoot-root/sucker fresh and dry weights, oil yield, total chlorophyll, total phenol and total sugar content of fresh leaves. In general there was a positive relationship between the initial inoculum levels of *M. incognita* and reduction in all the test parameters. The maximum reduction in corresponding parameters was 43.4, 45.0, 48.9, 45.7, 49.6, 42.5, 45.5, 47.0 and 45.9%, respectively, at the highest initial inoculum level (25,000 J2/5 kg soil) as compared to uninoculated control. There was a negative relationship between initial inoculum densities and rate of nematode multiplication. Maximum nematode final population Pf (1,33,430) and root-knot index (3.00) were observed at the highest Pi (25,000 J2/5 kg soil), whereas, maximum Rf (81.18) was observed at minimum Pi (500 J2/5 kg soil).

Increasing inoculum levels of *S. sclerotiorum* also exhibited a gradual increase in extent of reduction in, shoot height, shoot, roots/suckers, fresh and dry weight, oil yield, chlorophyll, total phenol and total sugar content of *M. arvensis* cv. Gomti, and increase in the percent roots/suckers infection by the fungus. The maximum reduction in the corresponding test parameters was 30.4, 39.8, 43.6, 40.3, 42.9, 28.9, 31.4, 34.8 and 31.6%, respectively at the highest initial inoculum level of 12 g fungal mycelium/5 kg soil as compared to uninoculated control. At the lowest Pi (1 g mycelium/5 kg soil), infection was observed 18.0% and at the highest Pi (12 g mycelium/5 kg soil), it was 80.2%.

The sequential, simultaneous and single inoculation of *M. incognita* (5000 J2/5 kg soil) and *S. sclerotiorum* (3 g mycelium/5 kg soil) on *M. arvensis* cv. Gomti indicates that the highest reduction in plant growth, and plant chemicals measured were found in plants inoculated with the nematode and fungus simultaneously followed by nematode inoculated seven days prior to fungus and fungus inoculated seven days prior to nematode, respectively. The maximum reduction in shoot height (32.6%), shoot dry weight (48.2%), root/sucker dry weight (51.5%), oil yield (33.3%), total chlorophyll (36.3%), total phenol (37.2%) and total sugar (32.3%) was observed in simultaneous nematode and fungus inoculation. Highest reproduction rate (19.71) of *M. incognita* and root-knot index (1.90) were observed in plants inoculated with the nematode alone, whereas, highest roots/suckers infection by the fungus (62.0%) was observed in plants inoculated simultaneously with the nematode and the fungus.

The effect of different pH levels (4.5, 6.0, 7.5, 9.5) on plants inoculated with nematode alone and nematode and fungus simultaneously showed maximum reduction in shoot height, shoot, root/sucker, fresh and dry weight, oil yield, chlorophyll, total sugar and total phenol content at pH 9.0 followed by 7.5, 6.0 and 4.5, respectively, whereas, the plants inoculated with fungus alone showed maximum reduction in corresponding parameters at pH 4.5 followed by 6.0, 7.5 and 9.0 respectively. The highest reproduction rate and root-knot index were observed at pH 9.0 followed by 7.5, 6.0 and 4.5, respectively. The roots/suckers infection due to *S. sclerotiorum* was found to be highest at pH 4.5 followed by pH 6.0, 7.5 and 9.0, respectively.

The effect of different soil types on various plant growth parameters of *M. arvensis* cv. Gomti was significant, both in the absence and presence of the nematode and the fungus. The highest reduction in plant growth and oil yield was observed in loamy sand soil followed by sandy loam, sandy clay loam and sandy clay, respectively as compared to uninoculated plants. The highest nematode population, reproduction rate, root-knot index was observed in loamy sand followed by sandy loam, sandy clay loam and sandy clay respectively.

The various treatments, viz. carbofuran (1.5 mg a.i./kg soil), carbendazim (1.0 mg a.i./kg soil), neem seed powder (50 mg/kg soil), *P. lilacinus/T. harzianum* (50 mg culture/kg soil) and *P. fluorescens* (50 mg/kg soil) used alone and in different combinations (half of the standard dose) resulted an increase in the growth of *M. arvensis* cv. Gomti in comparison to untreated inoculated plants. Greatest improvement in plant growth and reduction in reproduction of *M. incognita* was achieved in plants treated with neem seed powder + carbofuran and in descending order carbofuran alone, neem seed powder alone, carbofuran + carbendazim, *P. lilacinus* + neem seed powder, *T. harzianum* + neem seed powder, *P. fluorescencs* + neem seed powder, neem seed powder + carbendazim, *P. lilacinus* + *T. harzianum*, *P. fluorescencs* + *P. lilacinus*, *P. fluorescencs* + *T. harzianum*, *P. lilacinus* alone, *T. harzianum* alone, *P. fluorescencs* alone and carbendazim alone, respectively

All the treatments, viz. carbofuran (1.5 mg a.i./kg soil), carbendazim (1.0 mg a.i./kg soil), neem seed powder (50 mg/kg soil), *P. lilacinus/T. harzianum* (50 mg culture/kg soil) and *P. fluorescens* (50 mg/kg soil) used alone and in different combinations (half of the standard dose) against *S. sclerotiorum* revealed that all the treatments were able to boost the plant growth and provided the satisfactory reduction in disease incidence. The greatest plant growth and lowest root/suckers infection was observed in the plants treated with neem seed powder + carbendazim, followed by carbendazim alone, neem seed powder alone, carbofuran + carbendazim, neem seed powder + *T. harzianum*, neem seed powder + *P. fluorescens*, neem seed powder + *P. lilacinus*, neem seed powder + carbofuran, *P. fluoresens* + *T. harzianum*, *P. lilacinus* + *T. harzianum*, *P. fluorescens* + *P. lilacinus*, *T. harzianum* alone, *P. fluorescens* alone, *P. lilacinus* alone and carbofuran alone, respectively.

The management of *M. arvensis* cv. Gomti inoculated with *M. incognita* and *S. sclerotiorum* simultaneously showed that all treatments were able to improve the plant growth and oil yield as compared to untreated inoculated plants. These treatments were also able to reduce the root-knot disease development, nematode reproduction rate and roots and suckers infection due to

the fungus. The most satisfactory result were achieved by the neem seed powder (50 kg/ha) + carbofuran (1.5 kg a.i./ha), carbofuran (1.5 kg a.i./ha) + carbendazim (1.0 kg a.i./ha), neem seed powder (50 kg/ha) + carbendazim (1.0 kg a.i./ha).

The investigation conducted for the management of *M. arvensis* cv. Gomti in the field infested with *M. incognita* and *S. sclerotiorum* showed the positive efficacy of all the treatments against the disease complex. The greatest increase in shoot weight, oil yield and highest reduction in root-knot disease development, roots and suckers infection due to the fungus were provided by neem seed powder (50 kg/ha) + carbofuran (1.5 kg a.i./ha), carbofuran (1.5 kg a.i./ha) + carbendazim (1.0 kg a.i./ha), neem seed powder (50 kg/ha) + carbendazim (1.0 kg a.i./ha), carbofuran alone (3.0 kg a.i./ha), and neem seed powder alone (100 kg/ha).

The studies carriedout under the Ph.D. programme have generated knowledge on the occurrence of *M. incognita-S. sclerotiorum* disease complex of *M. arvensis*, effect of soil factors on disease development and effective management options. This information is not only of academic importance but also highly useful for mint farmers. On the basis studies, it is concluded that farmers should have information of their field soil infestation with pathogens, transplant disease-free suckers in main-field and if needed may adopt proper management options.

On the basis of the studies, application of neem seed powder (50 kg/ha) + carbofuran (1.5 kg a.i./ha) or carbendazim (1.0 kg a.i./ha) may be recommended for getting best results towards the management of *M. incognita-S. sclerotiorum* disease complex. However, to avoid the use of pesticides, application of neem seed powder (100 kg/ha) or neem seed powder (50 kg/ha) + *T. harzianum/P. lilacinus/P. fluorescens* (50 kg /ha) may be recommended.

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